BIOCHEMICAL VALUES IN NEWBORNS

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C E R T I F I C A T E

This is to certify that the work entitled "BIOCHEMICAL VALUES IN NEWBORNS", which is being submitted as a thesis for M.D. (Paediatrics) examination, 1992-93 of Bundelkhand University has been carried out by DR. SHALINI SHARMA in the department of Paediatrics, M.L.B. Medical College, Jhansi.

She has put in necessary stay in the department as per University regulations.

Dated 30 Sept, ,1992.

(RAMESH KUMAR) 30/9/92
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Dated : 30th Sept'92.

Shalini

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C O N T E N T S

INTRODUCTION

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Present century has ushered in an era of great biochemical advances. Strange though it may seem, practically every disease has now been shown to have some biochemical basis as the cause or its effect. The study of physiology and determination of normal values are mandatory for better management of patients. A diagnostic analysis is of only limited value to the clinician, if he does not know the normal value of substances. Moreover, the newborns are such patients, who rarely give any symptoms. To diagnose the diseased condition in the newborn wholly rests with the clinician. To aid in the diagnosis of certain diseased conditions, the normal value of substances in the blood/serum of newborns, provide an adjunct. In spite of this, only a few papers have been published on the normal values of clinically important components of blood serum, in newborns.

There are several reasons for the scarcity of data on the biochemical composition of blood and serum. When dealing with small children, analysis may sometimes necessitate employing more difficult micro-methods procedures in order to obviate taking huge blood samples.

Blood levels of certain constituents may be raised, or lowered as a manifestation of disease state. The extent of deviation from the normal range, which is needed to justify a confident diagnosis of abnormality, depends on the stability of normal figure.

Biochemical tests help in the recognition of morbid conditions of newborn and determine the prognosis/ survival of the infant during the crucial period following his birth. Thus, the study of biochemistry has of late gained an impetus, but the literature on the normal blood composition of Indian newborns is scarce and in a few studies that have been reported, the number of subjects investigated have usually been small.

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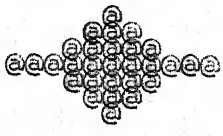
AIMS & OBJECTIVES

This study was performed, on cord blood with following aims and objectives :

1. To find out the normal biochemical values in newborn babies at birth.

2. To establish the relationship, if any, of these biochemical values with birth, weight and gestational age.



REVIEW OF LITERATURE

REVIEW OF LITERATURE

Dramatic changes in many biochemical, physiological and anatomic functions take place as a result of birth and continue during the first hour, days and even weeks of extrauterine life.

Biochemistry is the study of the chemical composition of living matter and the changes that occur in it. Such changes under normal conditions are termed physiological; abnormally they are pathological. Both phases are important. It is self evident that a knowledge of the normal should precede a consideration of the abnormal.

By normal value is meant that amount of a constituent present in the body fluids or that excreted by a healthy human being. In fact, this amount varies over a range, and is affected by a number of factors including age, sex, season, race, diet and genetic constitution. Thus, while most healthy persons can be included in a class having the accepted normal amount, some individuals are found to show divergent figure. Such exceptional individuals may be entirely normal in all other investigated aspects, and hence it is now recommended that the range should be termed 'reference' rather than normal.

At a very early stage in this work, wootlon, King and Maclean Smith (1951) and Wooton and King (1953)

investigated about 80 normal adults aged 20-50 years. These workers found that it was not always possible to express their results as an average normal figure with a standard deviation because only in a minority of cases could the values be fitted by a symmetrical normal distribution of Gaussian type. Most of the remaining skewed distributions were satisfactorily fitted by Lognormal curves of a type which have been used for biological data. It is thus necessary to give these normal values in terms of a range.

Finally, the normal range concept carries the implication that results outside it are abnormal and potentially harmful despite the convention that the normal range includes only 95 percent of normal people.

The difficulties of defining a normal range in a useful way have led to the concept of reference values (Alstrom et al, 1975). Reference values are the values of a particular quantity obtained from individuals under study. Reference values include all results from individuals in the defined state. The criteria used for the selection of these individuals must be stated, together with the specimen collection conditions. Thus data reported should include details of sex, age, race, posture, time of day, exercise etc., whether a tourmiquet was used and for how long, analytical method used and its performance.

If the total data are subjected to statistical treatment to exclude results at one or both ends of the reference range e.g. between the 25th and 97th percentiles the appropriate term is reference interval; but the statistical procedure should be stated and if a parametric method is used e.g. mean ± 2 standard deviations, it should be justified.

Blood glucose :

Before the introduction of techniques using glucose oxidase or o-toluidine the great majority of methods were done in three stages -

1. Precipitation of the blood proteins.
2. Reduction, either of an alkaline cupric copper solution to cuprous oxide, or of alkaline potassium ferricyanide to ferrocyanide.
3. Estimation of the amount of such reduction, either iodometrically or colorimetrically.

An important factor in connection with the determination of blood glucose has been the presence in blood of substances other than glucose which reduce these alkaline copper and ferricyanide reagents. One of the most important is glutathione, present in the red cells which contain the greater part of these substances. Other such

substances present in small concentration include threonine, uric acid & ascorbic acid. Glucoronic acid and its compounds also make an appreciable contribution. They are included in varying amounts in methods using the above reduction techniques upto an equivalent of about 30 mg glucose per 100 ml in the Folin-Wu procedure. (1.7 ml glucose per litre).

The method used to precipitate the proteins and the nature of the alkaline copper and ferricyanide reagents influence the amount included. Several workers found that if the blood is put into an isotonic solution of sodium sulphate in which the cells are not hemolysed, diffusible substances pass into solution and the cell envelops which contain much of the non-glucose reducing substances, are taken down with precipitate. Also precipitation of the proteins with heavy metal ions removes more of it than with tungestic acid. With the introduction of techniques, using glucose oxidase which oxidises glucose to gluconic acid and has very little effect on any other sugar, it was expected that true glucose values would be obtained. However, fluoride, protein precipitants, uric acid, glutathione and ascorbic acid have been said to influence the action of enzymes used in this technique (False et al, 1961).

The glucose oxidase method has been adopted for use with the Auto-Analyzer-Technique using the colour developed with o-toluidene. Technique using alkaline ferricyanide is also available. In the letter case, since dialysis removes the red cells, there is no interference from other substances but some low molecular weight substances which can reduce the ferricyanide, pass through the membrane so that the values are a little higher than those given by glucose oxidase method.

A different technique introduced relatively recently and originally due to Hultmann (1959) used the colour given by aldoses with o-toludine in glacial acetic acid. In the absence of other aldoses such as galactose and lactose, procedure using this reaction gives values as low as and even slightly lower than those using glucose oxidase and has proved simple and very suitable for routine use.

Glycolysis :

Glucose disappears fairly rapidly from whole blood on standing, so that upto about 10 mg/100 ml (0.5 mml/1) may be lost per hour at room temperature. This is due to its conversion to lactic acid $C_6H_{12}O_6 - 2\ C_3H_6O_3$ a process, known as glycolysis which occurs even in sterile blood. This can be prevented by adding sodium fluoride to the anticoagulant.

A mixture of sodium fluoride and potassium oxalate in the proportion of one part to three part will prevent any loss of glucose for two to three days. An excess, may, interfere with the estimation. A mixture containing 20 mg of the substance is used per 5 ml of blood sample.

Glycolysis results from cell metabolism. There is no loss of glucose from serum or plasma which is free from cells.

Choice of blood specimen :

There has been an increasing tendency in recent years to use plasma or serum. These, more accurately reflect the glucose content of extracellular fluids. Although the concentration of glucose inside the cells and in plasma is the same, yet because of the lower water content of cells (73 percent in the cells compared to 93 percent in the plasma) plasma glucose is higher than that of whole blood, roughly by 12-13 percent. This has been corrected for by multiplying the whole blood glucose concentration by a factor of 1.15 and adding 6 mg/100 ml (0.33 mmol/1) to give plasma or serum glucose, which is reasonably accurate if the hematocrit is normal. Changes in hematocrit, however, lead to changes in whole blood in the opposite direction : the lower the hematocrit as in anemia, more nearly the whole blood glucose is to the plasma glucose and vice versa, as in

polycythemia. According to Zalme and Knowles (1955) for each change in hematocrit of 10 units there is a change in opposite direction of blood glucose by 3.6 mg/100 ml (0.20 mmol/1).

Plasma has the advantage over serum that the blood can be added directly to a mixture of anticoagulant and preservative and plasma is then separated immediately. If serum is used it must be separated as soon as possible and in any case not later than 30 to 40 minutes after taking the blood.

On practical grounds capillary (Arterial) blood is generally used.

Non Protein Nitrogen :

The term non protein nitrogen in blood is used to include the nitrogen from all nitrogenous substances other than protein. In urine, not containing protein, it is equal to the total nitrogen. Urea is the most important of the substances which make up non protein nitrogen, others being uric acid, creatinine and creatine, aminoacids, ammonia and a small amount of residual or undetermined nitrogen, possibly including some peptides, as well.

Determination of serum-urea :

Urea is the main end product of protein metabolism in the body. Removal of amino group from amino acids, from which urea is formed, takes place in the liver.

Determination of urea is important not only in renal disease, but in a wide range of conditions in which the kidney is not primarily affected. Some techniques use the formation of ammonia from urea by the action of enzyme Urease. The ammonia formed has been determined by aeration into acid (Van Slyke and Cullen, 1914) and colorimetrically by nesslerisation or by the Berthelot reaction using phenol and hypochlorite. The colorimetric procedures are much more sensitive. For many years the urease nesslerisation was by far the most commonly used method, although trouble was experienced from turbidity, color instability and non-linear calibration. The application in 1960 of the reaction of ammonia with phenol and hypochlorite, first noted by Berthelot as long ago as 1859, gives a more sensitive technique with a more stable colour which obeys Beer'slaw. The increased sensitivity gives much less trouble from turbidity.

Urease is completely specific for urea which it hydrolyses to carbonic acid and ammonia, the former then decomposes to give carbon dioxide and a second molecule of ammonia.

$$NH_2.CO.NH_2 + H_2O \text{ — } NH_3 + NH_2.CO.OH \text{ — } 2\ NH_3 + CO_2$$

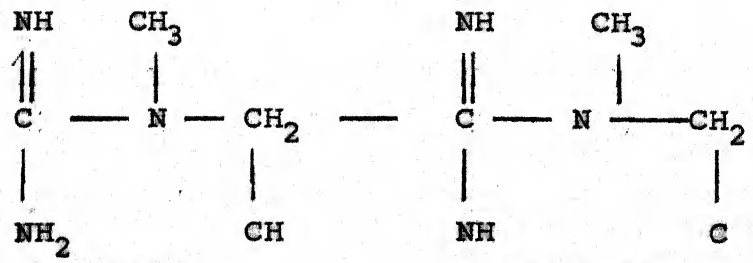
The enzyme acts optimally at 55°C and pH 7.0 to 8.0 and is inhibited by ammonia and fluoride.

A colorimetric method using the product formed when urea itself reacts with diacetyl was devised by Fearon (1939) but because of the non-linearity of the colour development and the need to heat with strong acid at near 100°C it did not achieve widespread use until adopted for the Auto-analyzer.

For manual use a urease method with nesslerisation or the Berthelot reaction is mostly used. Presently the workers prefer the latter method because of its greater sensitivity and better colour. Surveys indicate that the urease-nesslerisation method as practised gives low results especially at higher values. The use of low activity urease preparations and the non-linear calibration curve contributes to this. In these days, automated methods using almost exclusively some variant of diacetyl monoxime are most frequently used.

Creatine and Creatinine -

Creatinine is the internal anhydride of creatine methyl guanidoacetic acid, so these substances are related thus :-



Conversion of creatine to creatinine takes place readily on warming in acid solution. In a neutral solution of creatinine there is slow partial formation of creatine. Creatine is synthesized in the liver from which it passes into circulation to be taken up almost entirely by muscle in which it is converted into creatine phosphate (phosphocreatine). In muscle contraction energy is supplied by the conversion of ATP to ADP. Creatine phosphate is available as the source of a high energy phosphate bond for the immediate reformation of ATP by the action of creatine kinase. Resynthesis of ATP largely occurs during aerobic glycolysis and requires oxygen. The creatine phosphate thus provides additional synthesis of ATP during muscular contraction under anaerobic condition. Following aerobic glycolysis some of the ATP is used to reconvert creatine to creatine phosphate. There is some spontaneous conversion of creatine and creatine phosphate into creatinine; about 2 percent of the total, each day. This is related to the total muscle mass (roughly the body weight) and remains the same from day to day unless the muscle mass changes.

The two substances are handled differently by the kidney. Both are filtered at the glomerulus but whereas there may be some tubular excretion of creatinine, creatine is partly reabsorbed in the tubule and at low concentrations

in the plasma. This reabsorption may be so efficient that there is little or no creatine left in the urine.

Determination of Creatine and Creatinine :

The method commonly used for the estimation of creatinine makes use of the Jaffe reaction, the production of a red colour with an alkaline picrate solution. This reaction is not specific for creatinine and other substances in blood also give this colour. However, "chromogen" thus estimated and expressed as creatinine has clinical significance. Bonsnes and Taussky (1945) made a useful study of the best conditions for the development of this colour, a subject further studied by Koscoe (1953) and by Owen et al (1954). The latter workers also studied methods for determining true creatinine by the use of No-bacteria (corynebacteriun ureafaciens) which removes creatinine and by adsorption of creatinine on to Lcoyd's reagent. They concluded that in the case of serum upto 20 percent of the total chromogens could be non-creatinine substances but only upto 5 percent in the case of urine. Ralston (1955) also studied the use of Lloyd's reagent. In whole blood the percentage of non-creatinine substances is much higher than in serum which, therefore, should be used for the determination.

Slot (1965) observed that the colour given by creatinine faded rapidly after acidifying with sulphuric

acid, whereas that of non-creatinine chromogens remained. He used this to determine the creatinine by difference, reading again five minutes after adding the acid.

The Plasma Proteins :

The plasma proteins form an extraordinary complex mixture. Their functions, which are varied, can be summarised as follows :-

(a) Nutritive - The amino acids from the metabolism of protein enter the amino acid pool, part of which is used for the synthesis of new protein or other nitrogeous compounds while that not so used is determinated to give substances, which are either completely catabolised to carbon dioxide and water or used for the formation of glucose (gluconeogenesis).

(b) Control of body water distribution - The colloid osmotic pressure (oncotic pressure) of the plasma proteins counter-acts the hydrostatic blood pressure, thus maintaining the required circulating blood volume. Albumin, because of its higher concentration and lower molecular weight than most of the globulins, is the major contributor.

(c) Buffers - Proteins play a small part in maintaining the plasma pH. They are negatively charged at body pH and so act as bases, accepting hydrogen ions.

(d) Transporting agent - While albumin can transport a wide range of substances, other proteins carry only a particular one, e.g., transferrin carries iron. Important groups of substances, which are not transported include hormones, viz., cortisol and thyroxine and lipids, fatsoluble vitamins and metals. Some toxic substances are rendered harmless by binding to protein.

(e) In Blood Coagulation - Many of the factors thus involved are proteins.

(f) Protective - The immunoglobulins are antibodies which provide a defense against infection.

(g) As enzymes - The wide range of enzymes present in plasma are proteins.

Many plasma proteins, including albumin, fibrinogen and most globulins, are formed in the liver; immonoglobulins are produced by the reteculoendothelial system, the lymph nodules and plasma cells; the enzymes are released from various organs.

The plasma contains members of both the major groups of proteins, the simple and conjugated. Simple proteins, composed only of amino acids linked by peptids bonds, include albumin and some globulins, while conjugated proteins, containing other substances bound to

the polypeptide protein, embrace glycoproteins containing carbohydarates, lipoproteins containing lipids and metalloproteins containing iron, copper or zinc.

Determination of Plasma Proteins :

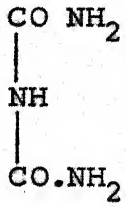
For many years the determinations most often performed were of total proteins in serum and of albumin after the globulins had been separated by salt fractionation.

The separation of serum proteins into albumin and globulins by this means was one of the first to be made. Albumin is soluble in water whereas globulins are not but are soluble in weak salt solution going into solution at concentration of electrolytes of about 0.1 mol/1. This phenomenon called "salting in" is due to electrostatic attraction between the salt ions and the charged groups of the protein molecule which decreases the attraction of the protein molecule for each other.

As the salt concentration increases, however, the salt ions compete for the water molecules of the hydrated polar groups of the protein so that these become dehydrated and the protein less soluble - the phenomenon of "salting out". Ammonium sulphate appears to have been first used for this purpose, half saturation precipitating the globulins but leaving the albumin in solution. Full saturation precipitated all the proteins.

The globulins are then obtained by difference. The most accurate technique was considered to be Kjeldahl digestion of proteins, followed by determination of ammonia formed, by distillation into acid, by nesslerisation or more recently by the Berthelot reaction. However, this is more time consuming and needs more care in execution than colorimetric methods which are simpler and more readily adoptable for larger batches. Of these, the most used has employed the biuret reaction.

Substances which contain two CO. NH_2 groups joins togather directly or through a single carbon or nitrogen atom and those which contain two or more peptide links, give a blue to purple coloured compound with alkaline copper solution. The reaction takes its name from the fact that the simple substance biuret gives the same kind of colour with cupric ions.



Biuret

One copper atom complexes with four molecules of biuret, the linkage being to the central nitrogen atom. It is thus given by proteins, the shade of colour being different with different proteins.

Other colorimetric methods use selective reactions with various reagents to detect individual amino acids present in proteins. The best known of these are those which use blue colour given by Tyrosine with Folin-ciocalten phenol reagent. This method (Greenberg and Merolubova, 1936) was once widely used, but suffers from the disadvantage that different proteins have very different tyrosins equivalents.

The biuret method in its more recent forms is preferred. Other techniques used to determine protein depend on light absorption in ultraviolet range, measurement of the refractive index, or the property of albumin to bind to a number of coloured substances (Martinek, 1970).

Serum Enzymes :

Blood serum contains a number of enzymes whose concentration varies with the changes in physiological state. These enzymes which catalyze the reactions of intermediate metabolism, are unevenly distributed and are concentrated or localized in specific cells of specific tissues. Some enzymes appear in the blood stream as a result of the normal processes of cellular distruction and removal. This increase in specific enzyme activity in the blood may indicate not only a pathological process but also identifies the specific tissue involved.

Serum enzyme levels are determined indirectly in terms of activity rather than concentration. The serum is mixed with the substrate on which the enzyme acts under conditions favourable to the reaction. After a given time period, the reaction is stopped by addition of an agent which stops the enzymic action (mineral or organic acid, heat, heavy metal ion, etc.) and the concentration of the product of the reaction or the remaining substrate is determined directly, if possible, or indirectly via the addition of some complexing agent to form a measurable coloured complex. Enzyme activity, so determined, is then expressed in terms of units which may be defined as the mg of substrate disappearing or as mg of a product appearing per unit time under the influence of a unit volume of serum.

Determination of Aminotransferases (Transaminases) :

Transamination is the term given to the process in which an amino group is transferred from an amino acid to Keto acid. As a result a different keto acids is formed. All naturally occuring amino acids can take part in such reactions; different enzymes are, however, involved. Two clinically important examples are L aspartate : 2 oxoglutarate aminotransferase (aspartate amino transferase, AST) and L-alanin; 2 oxoglutarate aminotransferase (alnine amino-transferase, ALT).

AST Catalyses the reaction

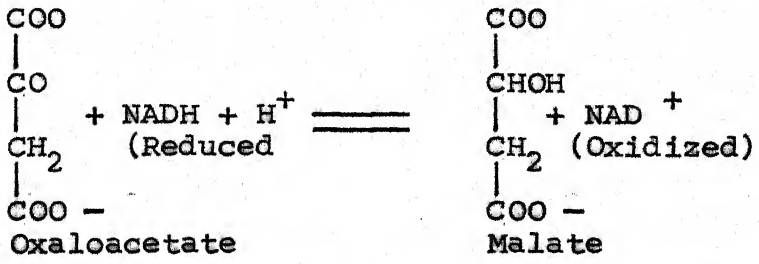
2 oxoglutarate + L Aspartate ⟷ L Glutamate + oxaloacetate

ALT catalyses the reaction –

2 oxoglutarate + L-Alanin ⟷ L-Glutamate + Pyruvate

Aminotransferases require pyridoxal -5- Phosphate as a cofactor. In most normal sera this is present in adequate amounts but it may be deficient in some pathological states leading to a reduced enzyme activity under the conditions used for its measurement.

Spectrophotometric and colorimetric techniques have been used for the determination of aminotransferases. For spectrophotometric method a coupled reaction using an enzyme requiring NADH to reduce the keto acid is employed (malate dehydrogenase in the case of Aspartate aminotransferase introduced by Karmen, 1955.



and Lactate dehydrogenase in the case of Alanine aminotransferase introduced by Wroblewski and Cabaud, (1957).

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{Lactate} + NAD^+$$

The reduced dinucleotide ($NADH_2$) absorbs radiation at 340 nm whereas the oxidized form does not. The unit of activity is expressed as the rate of decrease in absorbance (optical density) of $NADH_2$ per minute per ml. of serum at 340 nm.

Oxaloacetate is somewhat unstable, being gradually converted into pyruvate with loss of carbon-dioxide. Tonhazey et al (1950) using this method for determining AST activity in tissue extracts, added aniline citrate to catalyse this conversion so that the whole of oxaloacetate was converted to pyruvate within a short time. Cabaud et al (1956) adapted this technique for determining serum Aspartate aminotransferase activity. Their method involved protein precipitation and toluene extraction of the pyruvate 2, 4 dinitrophenyl hydrazone, before reading the colour.

Reitman and Frankel (1957) did not add aniline citrate. They used a pyruvate standard curve, itself standardised against the Karmen rate of reaction method.

Wootton (1964) used the technique of Reitman and Frankel.

King (1965) used oxaloacetate as standard while not adding aniline citrate and pyruvate.

Tietz (1970) used the same technique of Reitman and Frankel.

Berg meyer and Bernt (1974 b) also used the same method.

Calcium and Phosphorus :

Calcium is the most plentiful cation in the body. At all ages 99% of the body's calcium is in bones and teeth. Since the bones of infants are less densely mineralized than are those of adults, the body contents of calcium in infants and adults are significantly different, i.e. about 400 and 950 m Eq/kg of body weight respectively.

A small part of body calcium is present in plasma and other extracellular fluid. It is, however, vitally important in maintaining the correct conditions for normal neuromuscular transmission and glandular secretion, and for the activity of enzyme systems, particularly those involved in blood coagulation. There is almost no calcium in red blood cells and other intracellular fluids.

The average serum concentration of calcium is 10 mg/dL. Approximately 40% is protein bound, of which 80-90% is bound to albumin. The remaining 60 percent is

ultrafiltrable or diffusible. About 14% is complexed with anions such as phosphate and citrate, and the remaining 46% (4.8 mg/dL) is present as free ionic calcium. The ionized calcium is of greatest physiologic importance.

Although the total plasma calcium level varies little in health, the homeostatic mechanisms of the body are primarily directed towards maintaining a constant concentration of the ionized calcium fraction which is the biologically active component. Three hormones are involved in this process. They are : parathormone (PTH), a polypeptide produced by the parathyroids; calcitonin, a polypeptide produced by the interstitial or C cells of the thyroid; 1, 25 dihydroxychole calciferol (DHCC) produced from cholecalciforol by 25-hydroxylation in the liver followed by the action of 1 x hydroxylase in the proximal renal tubules.

The circulating calcium concentration is regulated at three sites :- bone, kidney and gut, the last two controlling exchange with the external environment.

Exchange of calcium between bone and extracellular fluid is of great importance and is much larger than the amount handled by the kidney or gut. Three processes contribute to this dynamic equilibrium ionic exchange, bone formation and bone resorption.

Bone mineral is deposited as minute crystals closely linked to the collagen fibres of osteoid, the uncalcified bone matrix. These small crystals have a large surface area in relation to their mass and this surface is bathed by interstitial fluid. Bone mineral is mainly hydroxyapatite arranged as a lattice of Ca^{++}, PO_4^{+++} & OH^- ions. Such ions in the lattice surface exchange rapidly with identical ions in the surrounding fluid, but it is possible for other ions of similar radius to replace one of the ions of hydroxyapatite. Thus Na^+ and H_3O^+ can replace Ca^{++} as can Mg^{++}, Sr^{++} and Pb^{++}. The skeleton thus contains quite a lot of sodium and contributes to the regulation of acid-base balance. Lead poisoning involves deposition in the skeleton and later release. The rapid ionic exchange of bone Ca^{++} and extracellular fluid Ca^{++} is quickly corrected if disturbed.

Bone is constantly remodelled even after growth cases. Bone destruction with mobilization of calcium and phosphate is achieved by the action of osteoclasts while formation of new bone involves production of osteoid by the osteoblasts and its calcification later-on.

Normally plasma Ca^{++} concentration is regulated by a negative feed back system. If the level falls

parathormone release is activated and in the presence of Dihydroxy chole-calciferol stimulates osteoclastic activity, thereby releasing Ca^{++} from bone to replenish the deficit. Alternatively, osteoclastic activity is reduced following suppression of PTH release, should the plasma Ca^{++} concentration attempt to rise above normal.

Several factors are involved in the deposition of mineral in osteoid dufing bone formation. These include concentrations of Ca^{++} and phosphate, blood pH and the presence of Dihydroxycholecalciferol. A useful biochemical marker of the activity of the osteoblasts is their production of bone isoenzyme "Alkaline phosphatase".

Calcium is mainly absorbed in the jejunum and for this process an adequate amount of Dihydroxychole-calciferol is needed to form a specific carrier protein in the mucosal cells (Wassermann and Taylor, 1968; Fraser and MacIntyre, 1970). The level of Dihydroxychole-calciferol is therefore the main factor regulating calcium entry into the body. Parathormone modulates the effect of dihydroxycholecalciferol since an increase in parathormone increases, the activity of 1 α - hydroxylase in the kidney thereby producing more dihydroxycholecalciferol. Other factors influencing calcium absorption include pH of

intestinal contents, extent of fat absorption, ratio of calcium to phophorus and presence of phytate.

In kidney, active tubular reabsorption amounts to about 98 percent. It is also under hormonal control. Parathormone increases the tubular reabsorption of calcium and diminishes that of phosphate, dihydroxycholecalciferol increases the tubular reabsorption of both ions while calcitonin has the opposite effect.

Determination of Serum Calcium :

For many years, the only method used was precipitation as oxalate, followed (after careful washing of the precipitate) by titration with permanganate or iodometric titration after adding excess permangate (Clark and Collip, 1925; Kramer and Tisdall, 1921; Sendroy, 1944). This method though reliable in competent hand is slow and requires 2 ml serum.

Present day techniques fall into two main groups, firstly, flame photometry (mainly as the atomic absorption technique) and secondly, those using the property of calcium ions to form coloured or fluorescent complexes with certain organic compounds. These latter were first used as indicator for complexiметric titration of calcium with such substances as ethylene diamine tetraacetate (EDTA) which has a greater affinity for

calcium than other complexing agents in serum. The end point was shown by the difference in colour of the free indicator and its calcium complex.

Now, more sensitive techniques have been developed using direct colorimetry or fluorimetry of serum treated with substances forming the appropriate coloured or fluorescent calcium complex. These organic substances are methylthymol blue and cresolphthalein complexone (calcein) for fluorimetric method.

PHOSPHORUS :

The body contains about 530 g phosphorus out of this 87 percent is present in the bones. There is phosphorus in many important substances, eg., in some proteins, lipids, nucleic acids and coenzymes. Phosphates also play a part in acid base regulation particularly by the kidneys.

The phosphorus of blood has been classified into -

1. Inorganic phosphorus, present as $H_2 PO_4^-$ and HPO_4^{--}
2. Organic or ester phosphorus such as glycerophosphate, nucleotide phosphate, hexosephosphate.
3. Lipid phosphorus, lecithin, cephalin, sphingomyelin.
4. A small amount of residual phosphorus.

The red cells are richer in phosphorus than the plasma mainly because they contain more ester phosphates.

Phosphate is absorbed from jejunum with calcium and is deposited in the skeleton or mobilized from it in a fixed proportion to calcium. Phosphate is excreted by the kidney following glomerular filtration and active tubular reabsorption. The latter process is inhibited by parathormone which increases phosphate excretion.

Determination of Inorganic phosphorus :

Most methods have used the reaction between phosphate and acid molybdate reagent. The hexavalent molybdenum of the phosphomolybdic acid formed absorbs light at 340 nm and can be reduced to give molybdenum blue. A variety of reducing agents has been used. Fiske and Subbarow (1925) used, 1,2,4 - amino-naphthol-sulphonic acid in a mixture of sodium bisulphite and sodium sulphite. However, the reagent does not keep well and is sensitive to the amount of acid present so that two different molybdate reagents are used. The reagent used for'Standard' contains more acid to compensate for the trichloracetic acid in the serum extract.

Kuttner and Lichtenstein (1930) introduced stannous chloride as reducing agent. The stock solution in hydrochloric acid keeps almost indefinitely and a deeper colour is obtained. The reagent can be used with smaller volumes of serum. However, the colour is unstable. p-Methyl aminophenol sulphate (Metol, Elon) introduced by Gomorri (1942) is more stable, is easier to prepare, is less affected by the amount of acid present and is not affected by oxalate, citrate or fluoride (if present in sample) but is less sensitive than the above reagents.

Itayi and Ui (1966) introduced an entirely new technique in which phosphate produced a colour change in a molybdic acid-dye solution. Malachite green was found to be the most suitable dye. Van Belle (1970) preferred methyl green to malachite green.

Robinson et al (1971) developed an automated method which did not use a reducing agent. They diluted the sample and dialysed that into dilute sulphuric acid. The sample was subsequently coupled with a molybdivanadate reagent to form a yellow complex and colorimetric reading was taken at 403 nm. This method proved less sensitive than reduction method.

Serum-bilirubin :

Since jaundice is due to an increase in the concentration of bilirubin in the blood, it is often, though not always connected with liver and biliary tract disease.

Physiology and Chemistry of bilirubin :

Bilirubin is formed from haemoglobin in the reticulo-endothelial system (Spleen, bone marrow and Kupffer cells in the liver). Biliverdin is first formed from the porphyrin part of the hemoglobin molecule while it is still attached to the globin. The α-methyne bridge (-CH=) is opened by oxygen. The biliverdin thus formed is then split off and the central of the three remaining bridges is reduced to a methene bridge ($-CH_2-$) to give bilirubin which then circulates in low concentration in the plasma, mainly attached to albumin (this bilirubin is not soluble in water but disolves in chloroform). It then enters the liver cells where it is transported, attached to unknown proteins disignated Y (Ligandin) and Z to reach the microsomes. Here it is conjugated by the action of glucoronyl transferase and is mainly converted to diglucoronide by ester formation (Cole and Lathe, 1953; Billing and Lathe, 1956; Schmid, 1959; Billing et al, 1957; Billing, 1959). Lathe (1956) suggested the terms bilirubin

and conjugated bilirubin for these two forms of bilirubin occuring in the body in place of old terms viz, haemobilirubin and cholebilirubin. The terms 'unconjugated' and 'Conjugated' for the free and ester forms respectively are used.

Conjugated bilirubin is water soluble but dissolves poorly in chloroform. It is actively transported from the liver cell into the bile capillary. The conjugated bilirubin passes along the bile ducts into the intestine. Here it is reduced by bacterial action and is also deconjugated (mainly in the colon) to urobilinogen and the terms used are d-uro-bilinogen, mesobilirubinogen and stercobilinogen. The greater part is excreted in the faeces as faecal urobilinogen. Some of the urobilinogen is absorbed from the intestines and passes into the portal circulation, back to liver. The greater part of this is re-excreted unchanged by the liver in the bile, a smaller part enters the general circulation and is excreted in the urine as urinary urobilinogen.

Determination of bile pigment in serum :

1. Van den Bergh Test : (Van den Bergh and Snapper, 1913)

Serum bilirubin is converted to purple compound azobilirubin when reacted with diazotized sulfanilic acid (Ehrlich's reagent). This reaction was first introduced in

1913 by Van den Bergh, who also noted that there were two distinct types of reactions : a fast or direct reaction; and a delayed or indirect reaction. The direct reacting conjugated bilirubin glucuronate is polar and water soluble because of the glucuronic acid moiety, and reacts within one minute with the aqueous diazo reagent to form the coloured azobilirubin complex. The unconjugated bilirubin is non-polar, soluble in chloroform but not in water. It is present in serum in colloidal suspension as a bilirubin-protein complex, and either does not react or reacts very slowly on long standing with the aqueous diazo reagent.

Both forms are alcohol soluble and when the reaction is carried out in methyl alcohol, the color, thus, formed represents total bilirubin. Indirect bilirubin is calculated as the difference between total and direct bilirubin.

2. Quantitative fractionation of serum Bilirubin - (Method of Malloy and Evelyn, 1937)

The principle is the same as that used in the qualitative Van den Bergh reaction. The azobilirubin formed is determined by photometric measurement.

3. Icteric Index -

The intensity of yellow pigmentation of serum is compared with a standard potassium bichromate solution.

The normal icteric index ranges between 4 & 6. The zone of latent jaundice (i.e. hyper-bilirubinemia without clinical signs of jaundice) is between 6 and 15. Above this value icteric symptoms may be observed. The yellow colour is considered to be due chiefly to the presence of bilirubin; on icteric index of 5 corresponding roughly to 0.1 to 0.2 mg of bilirubin per 100 ml of serum. The presence of certain other pigments will lead to errors, hemolysis is to be avoided.

Glucose Homeostasis in the newborn infants :

The difference between normal adult and neonatal blood glucose concentrations may be due to a variety or a combination of metabolic and endocrine factors.

Hypoglycemia :

The association between hypoglycemia and abnormal manifestations was first emphasized by Cornblath et al (1959), who suggested that brain damage suffered by some of the infants had been caused by hypoglycemia.

Causes of hypoglycemia in a newborn infant can be subdivided into :-

(i) Deficient hapatic glucose production,

(ii) Hyperinsulinism.

Deficiency of hepatic glycogen stores in utero, has been incriminated (Shelley and Neligan, 1966), but this probably is not the most important factor in producing hypoglycemia. The main energy source of normal infant switches from carbohydrate to lipid in the early hours of life. Hypoglycemia probably develops in small-for-date infant and in the normally developed baby, born very prematurely, because of deficient hepatic gluconeogenesis from lipids and amino acids, lack of substrate delivery, particularly lipid to the liver or to a combination of the two (Adam, 1971). Subnormal hepatic glucose production may also be responsible for hypoglycemia in rare metabolic disorders such as glycogen storage disease.

Hypoxia reduces the energy produced per unit of glucose and probably has an additive effect with hypoglycaemia, in the production of neuronal damage.

Hyperinsulinism is a cause of hypoglycemia in infants born to diabetic mothers. The frequency and severity of symptomatic hypoglycemia in these babies depends on the quality of maternal diabetic control.

Infants of gestational diabetics are less affected than those whose mothers are treated with insulin (McCann et al, 1966).

Babies born to women treated with oral sulphonylureas have a slight but very real chance of intractable hypoglycemia; for in addition to abnormal intra-uterine development they are born with therapeutic levels of an insulinotropic drug in the circulation (Kemball et al, 1970).

It is not generally appreciated that erythroblastosis fetalis is probably the second most common cause of hyperinsulinism (Milner, 1971) which may cause symptomatic hypoglycemia in the early hours of life or after exchange transfusion (Barret and Oliver, 1968; Schiff et al, 1971).

Rarer causes of hyperinsulinism are leucine sensitive hypoglycemia and abnormal development of the pancreatic islets.

Frank insulinomas (Garces et al 1968; Grant and Barbor, 1970; Schwartz and Zwiren, 1971) and disorganized development of the islets (Yakovac et al, 1971; Harken et al, 1971) are now being reported more frequently.

Hyperglycemia :

Hyperglycemia is rare in the neonate. It may occur following intravenous glucose infusion, exchange transfusion with acid and dextrose blood and in anencephalic baby due to poor utilization of glucose.

Neonatal infection may cause hyperglycemia. Transient neonatal diabetes mellitus may be seen in small-for-date babies.

Blood glucose values :

In full term babies :

Mekittrick (1940) has mentioned that the value of 40 mg percent of blood sugar should be regarded as normal in the first week of life.

Cornblath et al (1956) reported a mean blood glucose value of 60 mg percent at a body temperature of 97° to 98°F in this age period as compared to 45 mg percent when newborns were allowed to cool.

Compbell et al (1967) reported that at the age of 6 hours over 75 percent of all babies showed true glucose level of around 40 mg percent.

The average lower limit of normal blood glucose level is 30 mg/100 ml in term infants and 20 mg/100 ml in infants born prematurely (Cornblath and Schwartz, 1976).

In small for date babies :

In small-for-date babies, mean blood glucose value in the first week of life was 48.2 mg percent while the lowest mean value of 32 mg percent was encountered at the age of 13-18 hours.

Infants of low birth weight have a mean blood glucose level of 30 mg % during the first day of life (Cornblath et al, 1956). Values ranging between 39 to 47 mg percent were reported by Blum et al (1969).

Preterm newborns :

The preterm newborns showed a mean blood glucose value of 45 mg percent at birth which fell to a low mean value of 31.8 mg percent at the age of 13 to 18 hours (M. Bhalla et al, 1977).

Cornblath et al (1959), Brown and Wallis (1963) and Haworth (1965) reported that blood glucose during the first few days of life in premature babies ranged from 30 to 60 mg percent and levels less than 20 mg percent were regarded as abnormal.

Haworth (1965) and Watcher (1966) reported that concentration of 15-30 mg percent of blood glucose unaccompanied by clinical signs of hypoglycemia were often observed in individual babies.

Villee (1953) has shown that enzyme glucose - 6 - phosphate dehydrogenase makes its first appearance in the foetal liver by about 12 to 15 weeks and reaches its full activity by 10 to 24 weeks. From this period onwards the glycogen stores in the foetal liver increased gradually and reached normal adult concentration of 40 to 60 mg per gram liver tissue.

New borns liver carbohydrate concentration is about twice that of the adult and most of this is accummulated in the last three months of foetal life. The very premature are, therefore, apt to become hypoglycemic (Shelly, 1964).

Values of Blood urea :

It is seen that the mean value falls during the first few hours of life and then slowly rises and finally stabilizes as the kidneys begin to function.

A steady blood-urea level indicates that a balance exists between the forces of production and of excretion. A rise, therefore, in the first three days of life means that during this time the production of urea is exceeding its excretion.

In the first three days of life a baby takes little food hence the urea produced from the proteins in the milk will be immaterial. But red blood cells are being destroyed, and some urea is being formed from the breakdown of the proteins of some tissue to supply the needs of other more essential organs. A relatively large amount of urea may be coming from this source, for in the first few days of life babies are in a state of hydropenia and it exaggerates tissue breakdown (Schiff 1929, McCance 1936). There is abundant evidence, however,

that hydropenia will curtail the ability of a newborn child to excrete urea (Mc Cance and Young, 1941; Young and Mc Cance, 1942). By reducing the volume of urine and hence the urea clearance, hydropenia may produce a comparable rise of blood urea level in an adult (Black et al, 1944).

The rise in blood-urea should therefore, be attributed primarily to the effects of hydropenia on renal function. Fall in blood urea after the third day, in spite of the increased intake of protein food is primarily due to infant's return to a state of full hydration and consequently to increased urea clearances. Other reason could be that the kidney is relatively ineffecient for some time after birth, and it is possible that a rapid expansion in its functional capacity takes place in the first few days of extra uterine existence.

The initial fall in blood urea is probably due to utilization of urea for enzyme protein formation (Giordance, 1963; Houpt, 1963).

Serum urea increases in a number of diseases in addition to those in which the kidneys are primarily involved. These are :-

- Severe and protracted vomiting.
- Pyloric and intestinal obstruction.

- Prolonged diarrhoea.
- Pyloric stenosis with vomiting.
- Crises of Addison's disease.
- Shock due to -
  - severe burns,
  - acute haemorrhage,
  - post operative conditions.
- Fever & other toxic conditions.
- Cardiac failure.
- Mercurial poisioning.
- Hydronephrosis.
- Congenital cystic disease of kidneys.
- Renal tuberculosis.
- Stones in the urinary tract.
- Stricture of the urethra.
- Tumours of bladder

Decreases in serum urea is rare. It only indicate severe liver diseases.

Serum Creatinine levels in newborns :

Serum creatinine concentration is one of the simple and commonly used indices of the glomerular filtration rate. It is useful for early recongnition of deranged renal functions. Creatinine concentration is known to vary in the neonatal period and early infancy.

The values of serum creatinine is increased in -

- muscle disorders -
  - acute necrosis of muscle
  - progressive muscular dystrophy
- Starvation.
- Diabetes mellitus
- Fever
- Hyperthyroidism
- Vitamin E deficiency.
- Renal failure.

Reference ranges of serum creatinine are lacking in neonates. Stone street et al, 1978 in their study on low birth weight neonates noted a mean value of 1.3 $\pm$ 0.07 mg/dL which stabilished at 0.6 $\pm$ 0.05 mg/dL during the second and third months of life.

Serum creatinine levels were higher in preterms as compared to term infants indicating a lower glomerular filtration rate in them (Valia et al, 1981).

The values of serum creatinine were similar to those obtained by Walia et al (Rudd et al, 1983).

The value of serum creatinine obtained by Walia et al was confirmed by S. Awasthi et al in 1988.

Value of Total Protein and serum albumin in Newborns :

Only meagre information exists about the albumin and globulin levels in the blood of new-born human infants.

The plasma protein level increases in the first hour, due probably to loss of water from the plasma in the large calibre vein of the cord when compared with the plasma from capillaries as, with the changes of intravascular pressure in relation to tissue pressure caused by the haemodynamic change occuring soon after birth, the osmotic pressure of the plasma protein is insufficient to retain as much water as in utero. After this initial adjustment, the protein level is constant for the rest of the first 24 hours, quite possibly due to the haemoconcentration. Lewis and Wells (1922) reported that in 6 cord blood samples, they had examined, the euglobulin fraction was below normal. Their results and those of Boyd (1922) also suggest that the ingestion of colostrum is necessary for, and is followed by a rise in the euglobulin level. Darrow and Cary (1933) compared the total protein concentration in serum of full term and premature infants. They found a concentration of 5.52% in the full term and 4.9% in prematures.

Denzer, Reiner and Weiner (1939) found a concentration of 6.04% in full term infants.

Trevorrow et al (1942) who followed the albumin and globulin levels of normal full term infants from the time of birth until adult values were reached, reported a mean albumin level of 3.79 ± 0.33% at birth; beginning to rise after about a month until the adult level of 4.70 ± 0.32% was reached at 6 months to a year of age.

Hickman et al (1943) also reported figures for the total protein as 4 - 7% in full term infants and 3.7 - 5.4% in prematures, during the first four weeks of life.

Rapoport et al (1943) from a study of 17 premature infants (birth weight under 2 kg) and 17 full term babies confirmed the total serum protein of premature infants at 4.55% as against 5.11% in full term. In particular the low globulin value of 1.01 percent was seen in premature babies as against 1,34 percent in full term babies. They pointed out that globulin was still low in the infant at a time when albumin reached the adult level.

Remington and Bickford (1947) found the total protein concentration of 6.53 percent in normal full term. Mc Murray, Roe and Sweet (1948) has noticed total protein concentration of 6.0 percent in full term infants.

Graham, Wilson, Tsao, Baumann and Brown (1951) recorded a steady fall in total protein concentration from 6.12% at birth to 5.71% at 24 hours.

Gardner, Marks Roscoe and Breltell (1958) found total protein concentration of 6.5% Solomkin and Tauber (1959) noticed total protein concentration of 5.7% in their study. Oliver et al (1961) found total protein concentration of 6.1 percent at the end of 1 hour after birth.

A slight difference was observed in the albumin values of cord blood of premature babies and those of normal babies. There was a significant fall in total globulin in cord blood of all babies (premature and normal for gestation) as reported by T.C. Sitadevi (1968).

Values of inorganic phosphorus in newborns :

The blood phosphorus level is higher in infants and growing children than in adults. There is a small rise in phosphate level during the first few hours of life - possibly due to utilization of glycogen and the liberation of phosphate bound with glycogen. The later fall in phosphate is paralleled by a similar fall in calcium and may be due to deposition of calcium phosphate in the bones, but there are many factors causing variation in the phosphate level.

Serum Calcium values in newborns :

There is a progressive fall of calcium from birth till about 36 hours or occasionally longer. This fall is not universaly connected with the level of phosphate as has been suggested, since for much of the time both fall together. It is more likely due to a temporary dysfunction of the parathyroids. Value for calcium given by Bakwin (1937) was 11.0%. Denzer et al (1939) reported a value of 11.53%; Todd, Chuinard and Wood (1939) found the value of 11.27%. Bruck and Weintraub (1955) observed 10.59% while Acharya and Payere WW (1965) observed 9.34% serum calcium level in newborns.

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MATERIAL AND METHODS

MATERIAL & METHODS :

The present study was carried out in the Department of Pediatrics, in active collaboration with the Department of Obstetrics and Gynaecology, Maharani Laxmi Bai Medical College, Jhansi. Babies delivered in the labour room of Obstetrics and Gynaecology Department, between March, 1991 to February, 1992 were included in the study.

SELECTION OF CASES :

This consisted of 49 live newborn babies delivered by normal vaginal route. All singleton live newborns, irrespective of gestational age and birth weight were included in this study. Babies born to mothers with short stature and low weight (Height below 145 cm and weight below 40 kg), gross anaemia (Haemoglobin $\angle$ 8 gm % by sahlis method), edema, hypertension (Blood pressure exceeding 140 mm of Hg systolic or 90 mm Hg diastolic), congestive cardiac failure, evidence of perinatal stress, metabolic disease and blood group incompatibility were not included in this study. Also, babies born to mothers with a definite history of febrile episode in the third trimester and those having early rupture of membranes were excluded from the study.

Obstetrical History :

In each case, included in this study, date of last menstrual period was recorded. Gestational age was calculated in days and in completed weeks, from the first day of last menstrual period upto the time of delivery.

Natal and Post Natal history :

History was taken to record the mode of delivery. The other points noted were duration of labour, per vaginal bleeding, meconium staining of liquor amnil, cry and activity of the baby after birth and cyanosis; all to rule out any evidence of perinatal stress.

Examination of Newborn :

All normal newborns were included in this study. Thorough clinical examination was done in each case to exclude the possibility of congenital anomalies or infection. Anthropometric measurements viz., the head circumference, chest circumference, crown heel length were recorded in the working proforma. Birth weight of the newborn was recorded within one hour of the delivery.

The accurate assessment of gestational age was done by taking the history of last menstrual period. Gestational age in completed weeks was calculated from the first day of last menstrual period to the date of delivery.

Collection of blood sample :

For the estimation of Biochemical values, blood (5 ml) was collected from the cut end of umbilical cord, from the placental side, in the clean glass vial, with due precautions to avoid haemolysis and contamination with maternal blood. All glassware used in the study was thoroughly washed with distilled water and sterilized in hot air oven. Blood sample was allowed to cool at room temperature. After 2-4 hours, serum was separated using a pippette and then serum was centrifuged at 1000 r p m for 15-20 minutes. After centrifugation 2 ml of clear serum at the top of sample was transferred to another dried glass vial.

All samples were stored at -20^{o}C in a deep freezer. The samples were analysed for Biochemical values at a later date, with the help of commercially available kits. The experimental work was carried out in the Biochemical and Immunology Research Laboratory of the Department of Pediatrics.

Method of Estimation :

1. Method for glucose estimation in the serum :

Serum glucose was determined by using end point O-toluidine method.

Principle :

The method uses O-toluidine as a colour reagent (Hultman, 1959). O-toluidine reacts with the aldehyde group of glucose, in hot acetic acid solution to form an equilibrium mixture of glucosylamine and corresponding Schiff's base. The blue green coloured end product has maximum absorption at 630 nm. Intensity of colour developed is proportional to the original concentration of glucose present in the serum.

Reagents and Material used :

1. Commercially prepared kits for glucose estimation.
2. Reference serum.
3. Auto pippette.
4. Waterbath.
5. Colorimeter.

Procedure :

1. First 3 ml glucose reagent was taken in test tubes labelled as B-Blank, S-Standard and T-test (T_1, T_2, T_3 .....T_{49} unknown).
2. 50 ul distilled water was added to Blank test tube, 50 ul glucose standard was added in S-test tube and 50 ul of test serum was added in each T-Test tubes (T_1, T_2, T_3 .....T_{49}).

3. Then, contents of the test tube were mixed by lateral shaking and put into vigorously boiling water bath (100°C) for exactly 9 minutes. Tubes were quickly removed and cooled to room temperature by placing in cold water for 3 minutes.

4. Contents of tubes were transferred to cuvettee and one by one absorbance (optical density) of light by the solution (adjusted against the blank taken as zero at 620 nm) was read.

5. Concentration of glucose in the unknown (serum) was calculated by using the formula :

$$\frac{RT}{RS} \times 100 \text{ mg/dL} = \text{concentration of glucose}$$

where RT - Optical density (O.D.) of unknown serum.

RS - Optical density (O.D.) of standard.

2. Method for urea Estimation in the serum :

Urea was determined by using end point DAM (Diacetyle monoxime) method. For this commercially prepared kit was used.

Principle:

Urea reacts with diacetyle monoxime in acidic medium at 95°- 100°c to give pink coloured complex. Ferric ions are used to oxidise hydroxylamine formed in the reaction. Absorbance of pink coloured end product is measured at 520 ± 15 nm. The intensity of colour developed is proportional to the concentration of urea present in the serum.

Reagents and Material used :

1. Commercially prepared kit for urea estimation.
2. Reference serum.
3. Auto pippette.
4. Waterbath.
5. Colorimeter.

Procedure :

1. First 1.5 ml urea colour reagent A was taken in test tubes labelled as B-Blank, S-standard and T-test (T_1, T_2, T_3 .....T_{49}).
2. Next 1.5 ml urea colour reagent B was added to all test tubes.
3. Further, 20 ml urea Acid Reagent C was added to all the test tubes.

4. Finally 50 ul of distilled water was added to B-test tube; 50 ul urea standard was poured in S-test tube and 50 ul serum was pipetted in T-test tubes.

5. Contents of test tube were mixed by lateral shaking and kept in vigorously boiling water bath for exactly 10 minutes. The tubes were removed and cooled to room temperature by placing in cold water for 3 minutes.

6. Optical density of each test tube was measured at 520 nm against the blank adjusted to zero.

7. Concentration or urea in serum was calculated by the following formula :

$$\frac{RT}{RS} \times 40 \text{ mg/dL} = \text{Concentration of urea.}$$

RT - Optical density of unknown serum. (O.D.)

RS - Optical density of standard. (O.D.)

3. Method for creatinine estimation in the serum :

Creatinine was determined by using Jaffe-reaction. for this commercially prepared kit was used.

Principle :

Serum proteins are denatured by heat. Creatinine is eluted from the sample by heating with distilled water. Creatinine then reacts with picrate ions in alkaline solution producing red tautomer of creatinine picrate.

Reagents and Material used :

1. Commercially prepared kit for creatinine estimation.
2. Reference serum.
3. Auto pippette
4. Waterbath.
5. Colorimeter.

Step - I :

(a) Preparation of working standard solution.
For this 0.1 ml of stock standard was diluted by adding 10 ml of distilled water.

(b) Deproteinization of test sample was done by mixing -
Serum - 1.0 ml,
Distilled water - 1.0 ml and
Picric Acid - 6.0 ml.

The mixture was mixed well, kept in boiling water bath exactly for one minute and cooled immediately under tap water and centrifuged.

Step - II : Colour Development :

(c) 4 ml of supernatant was taken in each test tube labelled as T-test (T_1, T_2, T_3 .....T_{49}). Next 1.0 ml working standard was taken in test tube labelled as S-standard and 1.0 ml distilled water water was taken in B-Blank test tube.

(d) 3.0 ml picric acid solution was then added to B-tube and S-tube.

(e) Finally 1.0 ml Alkali reagent was poured into B-tube, S-tube and to each T-tubes (T_1, T_2, ...T_{49}).

(f) Contents of the test tube were mixed well and allowed to stand at room temperature for 20 minutes before measuring the optical density of B-Blank, S-standard and Test (T) against distilled water adjusted to zero at 520 nm.

(g) Concentration of creatinine in the serum was calculated by the formula :

$$\frac{\text{O.D. of test - O.D. of Blank}}{\text{O.D. of Standard - O.D. of Blank}} \times 3.0 \text{ mg/dL} = \text{Concentration of creatinine (mg\%) in test serum.}$$

4. Method for total Protein Estimation in the serum :

Principle :

Proteins and peptides containing atleast 2 adjacent peptide bonds react with cupric ions in alkaline solution forming violet coloured complex having maximum absorption at 540 nm. The concentration of low molecular weight peptides is too less to interfere.

Reagent and Material used :

1. Commercially prepared kit for total protein estimation.
2. Reference serum.
3. Autopipette.
4. Incubator.
5. Colorimeter.

Procedure :

1. First, 3 ml 'Total protein' reagent was taken in test tubes labelled as B-Blank, S-standard and T-test (T_1, T_2, T_3 .....T_{49}).
2. Next 50 ul distilled water was added to Blank test tube, 50 ul total protein standard to S-standard test tube and 50 ul reference serum in all T-test tubes (T_1, T_2, T_3 .....T_{49}).
3. Contents in each test tube were mixed by lateral shaking and then incubated at 37°C for exactly 10 minutes.
4. Optical density of all the test tubes was measured at 540 nm against blank adjusted to zero.
5. Concentration of total proteins in the serum was calculated by the formula :

$$\frac{RT}{RS} \times 6 \text{ gm/dL} = \text{Total Protein (mg\%)}$$

Where RT= O.D. of unknown

RS= O.D. of standard

5. Method for serum - albumin estimation :

Principle :

Serum albumin binds certain dyes such as bromocresol green forming colored complexes. The blue green complex thus formed has maximum absorbance at 630 nm, wave length. Concentration of albumin in serum is obtained by comparing the intensity of colored solution of unknown to a known albumin concentration solution.

Reagents and Material used :

1. Commercially prepared kit for serum albumin estimation.
2. Reference serum.
3. Autopippette and test tubes.
4. Colorimeter.

Procedure :

1. One test-tube as B-blank and one as S-standard was taken. Other test tubes were labelled as T_1, T_2, T_3 .....T_{49}.
2. 4.0 ml albumin reagent was taken in all test tubes.
3. 20 ul distilled water was taken in blank test tube and 20 ul albumin standard was taken in standard test tube. Then 20 ul reference serum was added in all other test tubes viz., T_1, T_2, T_3 .....T_{49}.

4. Mixing was done by lateral shaking and test tubes were allowed to stand at room temperature for 5 minutes.

5. Optical density of all test tubes was measured at 630 nm wave length against blank adjusted to zero.

6. Concentration of serum albumin in reference serum was calculated as under:

$$\frac{RT}{RS} \times 4 \text{ gm/dL} = \text{concentration of albumin (gm \%)}$$

RT = Optical density of unknown (O.D.)

RS = Optical density of standard (O.D.)

6. Method for serum calcium estimation :

Principle :

Calcium in serum reacts with O-cresolphthalein complexone in alkaline medium forming purple colored complex which is measured by colorimeter using yellow filter (wave length 570 nm).

Reagent and material used :

1. Commercially prepared kit for serum calcium estimation.
2. Reference serum.
3. Autopippette and test tubes.
4. Colorimeter.

Procedure :

1. Test tube labelling was done, one each for blank (B) and standard (S). Other test tubes were labelled as T_1, T_2, T_3, ...T_{49} representing test material.
2. 3.0 ml calcium color reagent was taken in all test tubes.
3. 1.0 ml AMP buffer was added in all test tubes.
4. Then o.05 ml reference serum was taken in all T_1 to T_{49} test tubes and 0.05 ml working color standard in S-standard test tube.
5. Mixing was done by lateral shaking and all tubes were allowed to stand at room temperature for 5 minutes.
6. Optical density of test tubes was measured against distilled water adjusted to zero at 570 nm wave length on the colorimeter, using yellow filter.
7. Concentration of serum calcium in reference serum was calculated as under :

$$\frac{\text{O.D. (Optical Density) of test-O.D. of Blank}}{\text{O.D. of standard - O.D. of Blank}} \times 10\text{mg}/100\text{ml}$$

= Concentration of serum calcium in test serum (mg %).

7. Method for serum inorganic phosphorus estimation :

Principle :

Test is based on 'modified metol method. Ammonium molybdate under acidic conditions reacts with phosphorus to form phosphomolybdate complex which reduces to blue colour developed is proportional to inorganic phosphorus concentration.

Reagents and material used :

1. Commercially prepared kit for inorganic phosphorus estimation.
2. Reference serum.
3. Test tubes and autopippette.
4. Colorimeter.

Procedure :

1. Test tube labelling was done as follows :

   B-for blank, S - for standard and T_1, T_2 ...T_{49} for unknown serum samples.

2. 1.0 ml catalyst reagent was taken in all, test tubes.
3. 1.0 ml molybdate reagent was added in all tubes.
4. 0.1 ml distilled water was added to B-blank, and standard in S tubes and 0.1 ml reference serum was added to all T-test tubes.

5. Finally, 1 ml of metol reagent was added to all the test tubes.

6. Mixing was done by lateral shaking and all tubes were allowed to stand at room temperature for 5 minutes.

7. Absorbance of unknown (T) and standard (S) against blank (B), adjusted to zero on colorimeter with a red filter, was measured within 30 minutes.

8. Concentration of unknown inorganic phosphorus was calculated as under :-

$$\frac{\text{Optical Density (O.D.) of unknown}}{\text{O.D. of Standard}} \times 5 \text{ mg/dL.}$$

= Concentration of inorganic phosphorus in mg/dL

8. **Method for serum bilirubin estimation :**
(Malloy and Evelyn method)

Principle :

Direct (conjugated) bilirubin couples with diazotized sulfanilic acid forming azobilirubin, a red purple colored product in acidic medium.

Indirect (unconjugated) bilirubin is diazotized only in the presence of its dissolving solvent (methanol). Thus red purple colored azobilirubin produced in the presence of methanol represents both direct and indirect fraction of total bilirubin. Difference of total and direct bilirubin is indirect bilirubin. Intensify of red purple color so

developed is measured, colorimetrically and it is proportional to the concentration of appropriate fraction of bilirubin.

Reaction can be represented as Under :

Bilirubin + Diazotized sulfanilic acid

|

Azobilirubin

(red purple complex)

Reagents and material used :

1. Commercially prepared kit for bilirubin estimation.
2. Reference serum.
3. Test tubes and autopippette.
4. Colorimeter.

Procedure :

1. For one reference serum 4 test tubes were taken and labelled as T_1 (total), T_2 (total blank), D_1 (Direct) and D_2 (Direct blank).
2. 0.2 ml serum was taken in all test tubes.
3. 1.8 ml distilled water was added in all the tubes.
4. 0.5 ml bilirubin diazoblank was added to T_2 and D_2 tubes and the 0.5 ml diazo working reagent was added to T_1 and D_1 tubes.
5. 2.5 ml distilled water was added to D_1 & D_2 and 2.5 ml methanol in T_1 and T_2 tubes.

6. All tubes were mixed well by lateral shaking and optical density of D_1 and D_2 tubes was read after one minute by using distilled water adjusted to zero at 540 nm.

7. T_1 and T_2 tubes of all reference sera were kept in dark at room temperature for 30 minutes. After that, optical density of both sets of tube (T_1 & T_2) were measured at 540 nm against distilled water adjusted to zero.

8. Optical density of artificial bilirubin standard was measured against distilled water adjusted to zero at 540 nm.

9. Concentration of bilirubin was calculated as follows :

$$\text{Total (A) Bilirubin (mg \%)} = \frac{\text{Optical density (O.D.) of } T_1 - \text{OD of } T_2}{\text{O.D. of S}} \times 10$$

$$\text{Direct (B) Bilirubin (mg \%)} = \frac{\text{OD of } D_1 - \text{O.D. of } D_2}{\text{O.D. of S}} \times 10$$

$$\text{Indirect Bilirubin (mg \%)} = A - B$$

9. **Method for SGPT estimation :**

(End point DNPH method)

Principle :

Transamination is the process by which an amino group of amino acid is transferred to an alphaketo acid with the formation of keto acid corresponding to original amino acid. The pyruvic acid formed in the reaction reacts with dinitrophenylhydrazine to give pyruvate dinitrophenyl hydrazine. The yellow colour is modified to intense brown by the addition of sodium hydroxide and optical density is measured colorimetrically at 520 nm.

Reagent and material used :

1. Commercially prepared kit for SGPT estimation.
2. Reference serum.
3. Autopippette and test tubes.
4. Water bath.
5. Colorimeter.

Procedure :

1. 1 ml of solution 'A' was taken into a tube and Placed in 37°c water bath for 5 minutes.
2. To this 0.2 ml of serum was added and mixed by lateral shaking and was kept in water bath for 30 minutes.
3. All tubes with different samples of test serum (solution 'A' added) were removed from water bath and 1 ml of solution B (DNPH) was added to each

one. Tubes were kept for 20 minutes at room temperature.

4. Next 10 ml of diluted solution C (0.4 N NaoH) was added to each test tube.

5. Mixing was done by lateral shaking and tubes allowed to stand at room temperature for 10 minutes.

6. Absorbance of solution was read at 520 nm wave length (using green filter) against water blank adjusted to zero.

7. SGPT value was calculated as per the standard curve, prepared as follows :

Preparation of standard curve :

1. Caliberation standard and distilled water was taken in 5 test tubes as follows -

   1.0 ml solution A - in test tube no. 1

   0.9 ml solution A - in test tube no. 2

   0.8 ml solution A - in test tube no. 3

   0.7 ml solution A - in test tube no. 4

   0.6 ml solution A - in test tube no. 5

2. Then solution D was added to test tubes as follows -

   0.1 ml in no. 2 test tube

   0.2 ml in no. 3 test tube

   0.3 ml in no. 4 test tube

   0.4 ml in no. 5 test tube

3. After this 0.2 distilled water was added in all tubes.

4. 1 ml of solution B (DNPH) was added in each tubes, mixed and allowed to stand for 20 minute exactly, at room temperature.

5. After 20 minutes 10 ml of solution C (diluted sodium hydroxide) was added to all tubes and kept for 10 minutes. Readings were taken against distilled water blank at 520 nm.

6. Standard curve was prepared plotting percentage absorption against unit per ml of SGPT.

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OBSERVATIONS

OBSERVATION

A study to determine the normal serum level of certain biochemicals in cord blood of newborn babies and their relationship to gestational age, birth weight was carried out on 49 newborn babies delivered at M.L.B. Medical College Hospital, Jhansi between March, 1991 to February, 1992. Relevant clinical features were noted, birth weight was recorded and gestational age was assessed from the history of last menstrual period.

Newborn babies were divided into two groups on the basis of sex. There were 25 (52.03%) male and 24 (48.97%) female cases in the study group (Table - 1).

Table - 1

Distribution of cases according to sex

Sl. No.	Sex of baby	No.of cases	Percentage
1.	Male	25	52.03
2.	Female	24	48.97
	Total	49	100.00

Moreover, cases were classified, as per WHO criteria (1961), into two birth weight groups. Newborns having birth weight leas than 2500 gms were labelled as Low Birth Weight (LBW) babies and included 10 (20.04%) cases, while newborns, with birth weight of 2500 gms or more were designated as normal weight babies, which included 39 (79.96%) cases. (Table-2)

Table - 2

Frequency distribution of cases according to birth weight groups

Sl. No.	Birth weight (gms)	No. of cases	Percentage
1.	∠ 2500	10	20.41
2.	2500 - 3000	33	67.35
3.	7 3000	06	12.24
	Total	49	100.00

Newborn babies were classified, according to gestational age into preterm (∠ 37 weeks); full term (37-41 weeks); Post term (7 41 weeks) groups. There were 42 (85.71%) cases in the full term group, 5 (10.20%) cases in pre-term group and 2 (4.08% cases in the post term group (Table-3).

Table - 3

Distribution of cases according to gestational age

Sl. No.	Group	No.of cases	Percentage
1.	Pre-term	05	10.20
2.	Full term	42	85.71
3.	Post term	02	04.08
	Total	49	100.00

Figures in parantheses indicate percentage.

Serum glucose values :

Table - 4

Showing mean ± S.D. values of glucose (mg / dL), sex-wise

Sl. No.	Sex of baby	No.of cases	Glucose (Mean ± S.D.)
1.	Male	24	61.05 ± 34.86
2.	Female	21	67.19 ± 37.12

't' = 0.55

'p' = $\angle$0.6

d.f. = 43

d.f. = degree of freedom

Table 4 denotes the mean value of glucose, as observed in different sexes of the study group. It was observed that mean cord blood glucose was higher in female (67.19 mg%) than male babies (61.05 mg%).

Table - 5

Showing mean ± S.D. Glucose in different birth weight groups (mg/dL)

Sl. No.	Birth weight (gms)	No. of cases	Glucose (Mean ± S.D.)
1.	⟨ 2500	09	57.80 ± 29.68
2.	2500 - 3000	33	62.74 ± 36.22
3.	⟩ 3000	03	95.20 ± 32.25

On comparison of mean serum glucose value in LBW babies with normal weight babies, it was observed that cord blood glucose was lower in the LBW group 57.80 ± 29.68 than in the normal birth weight group (65.45 ± 45.84).

Table - 6

Showing mean ± S.D. values of glucose in different gestational age groups

Sl. No.	Group	No. of cases	Serum Glucose (Mean ± S.D.)
1.	Pre term	05	82.31 ± 34.84
2.	Full term	38	61.97 ± 29.94
3.	Post term	02	76.05 ± 33.95

The overall mean glucose value observed during this study was 53.08 ± 22.84. The mean glucose values observed for various gestational age groups i.e. pre-term, full term and post term were 82.31 ± 34.84, 61.97 ± 29.94 and 76.05 ± 33.95 respectively.

Serum - Urea values :

Table - 7

Showing mean ± S.D. values of Urea (mg/dL); sex-wise

Sl. No.	Sex	No. of cases	Urea (Mean ± S.D.)
1.	Male	24	23.08 ± 12.60
2.	Female	24	24.53 ± 10.91

't' = 0.61

'p' = $\angle$ 0.6

'd.f.' = 46

Table 7 denotes the mean value of urea, as observed in different sexes of the study group. There was not much difference in the mean values of urea, between male (23.08 mg%) and female babies (24.53 mg%).

Table - 8

Showing mean ± S.D. urea in different birth weight groups (mg/dL)

Sl. No.	Birth weight (gms)	No. of cases	Urea (Mean ± S.D.)
1.	⟨ 2500	09	26.25 ± 09.13
2.	2500-3000	36	23.48 ± 10.43
3.	⟩ 3000	03	31.23 ± 09.52

On comparison of the mean values of serum-urea in the low birth weight (LBW) babies (birth weight ⟨ 2500 gm) with normal weight babies (birth weight ⟩ 2500 gm) table - 8, it was observed that cord blood urea values were higher in the low birth weight group (26.25 mg) in comparison to normal weight group (23.48 mg%).

Table - 9

Showing mean ± S.D. values of urea levels in various gestational age groups (mg / dL)

Sl. No.	Group	No. of cases	Serum - Urea (Mean ± S.D.)
1.	Pre-term	05	23.19 ± 10.39
2.	Full term	41	24.82 ± 10.62
3.	Post term	02	21.00 ± 03.00

The overall mean urea value observed during this study was 21.08 ± 11.00 mg %. The mean urea values observed for various gestational age groups i.e. pre-term, full term and post term were 23.19, 24.82 and 21.00 respectively.

Total serum protein values :

Table - 10

Showing mean ± S.D. values of total protein (g /dL), sex-wise

Sl. No.	Sex	No. of cases	Total Protein (Mean ± S.D.)
1.	Male	25	4.01 ± 0.60
2.	Female	24	4.11 ± 1.08

't' = 0.4

'p' = $\angle$ 0.7

d.f. = 47

Table 10 denotes the mean value of total protein as observed in different sexes of the study group. It was observed that mean value of cord blood total protein was higher in female (4.11 gm%) than male babies (4.01gm%).

Table - 11

Showing mean ± S.D. total protein in different birth weight groups (mg/dL)

Sl. No.	Birth weight (gms)	No. of cases	Total Protein (Mean ± S.D.)
1.	∠ 2500	10	3.54 ± 0.46
2.	2500 - 3000	36	4.32 ± 0.66
3.	⁊ 3000	03	4.06 ± 0.41

On comparison of the mean total serum protein value in low birth weight babies with normal weight babies (table - 11), it was observed that cord blood total protein value was lower (3.54 mg%) in the low birth weight group than in the normal birth weight group (4.32 mg%).

Table - 12

Showing mean ± S.D. values of total protein level in various gestational age groups (g / dL)

Sl. No.	Group	No.of cases	Serum-total-protein (Mean ± S.D.)
1.	Pre-term	05	3.32 ± 0.30
2.	Full term	42	4.25 ± 0.67
3.	Post term	02	4.00 ± 0.00

The overall mean total protein value observed during this study was 4.14 ± 0.69. The mean total protein values observed for various gestational age groups i.e. pre-term, full term and post term were 3.32 ± 0.30, 4.25 ± 0.67 and 4.00 ± 0 respectively.

Serum-albumin values :

Table - 13

Showing mean ± S.D. values of serum - albumin (g / dL), sex-wise

Sl. No.	Sex	No.of cases	Albumin (Mean ± S.D.)
1.	Male	25	3.72 ± 0.56
2.	Female	23	3.72 ± 1.31

Table 13 denotes the mean value of serum albumin in different sexes of the study group. There was no difference in the mean value of albumin between male (3.72 gm%) and female babies (3.72 gm%).

Table - 14

Showing mean ± S.D. albumin in different Birth Weight Groups (g/dL)

Sl. No.	Birth weight	No.of cases	Albumin (Mean ± S.D.)
1.	< 2500	10	3.30 ± 0.35
2.	2500 - 3000	35	3.91 ± 0.58
3.	> 3000	03	3.85 ± 0.31

On comparison of the mean serum value of albumin in the low birth weight babies with normal weight babies (table - 14), it was observed that cord blood albumin values was lower (3.30 mg%) in the low birth weight group than in normal birth weight group (3.91 mg%).

Table - 15

Showing mean ± S.D. values of serum albumin levels in various gestational age groups (g / dL)

Sl. No.	Group	No.of cases	Serum albumin (Mean ± S.D.)
1.	Pre-term	05	3.15 ± 0.43
2.	Full term	41	3.80 ± 0.61
3.	Post term	02	4.00 ± 0.00

The overall mean albumin values observed during this study was 3.65 ± 0.45. The mean serum albumin values observed from various gestational age groups i.e. pre-term, full term and post term were 3.15 ± 0.43, 3.80 ± 0.61 and 4.00 ± 0 respectively. It is clear from table 15 that serum albumin values increased with maturity.

Serum-calcium values :

Table - 16

Showing mean ± S.D. values of serum-calcium (mg/dL), sex-wise

Sl. No.	Sex	No.of cases	Calcium (Mean ± S.D.)
1.	Male	22	11.93 ± 3.70
2.	Female	23	12.85 ± 5.04

't' = 0.68

'p' = $\angle$ 0.5

d.f. = 43

Table 16 denotes the mean value of serum - calcium in different sexes of the study group. It was observed that cord blood calcium level was higher in female (12.85 mg%) than in male babies (11.93 mg%).

Table - 17

Showing mean ± S.D. calcium in different Birth Weight Groups (mg/dL)

Sl. No.	Birth weight (gm)	No.of cases	Calcium (Mean ± S.D.)
1.	∠ 2500	08	11.20 ± 4.97
2.	2500 - 3000	34	12.90 ± 3.19
3.	⁊ 3000	03	09.96 ± 5.42

On comparison of the mean of serum-calcium value in the low birth weight babies with normal weight babies (table - 17), it was observed that cord blood calcium level was lower (11.20 mg%) in low birth weight group than in normal weight group (12.90 mg%).

Table - 18

Showing mean ± S.D. values of serum calcium levels in various gestational age groups (mg / dL)

Sl. No.	Group	No.of cases	Serum-calcium (Mean ± S.D.)
1.	Pre-term	04	10.80 ± 4.33
2.	Full term	39	12.44 ±3.81
3.	Post term	02	14.95 ± 1.60

The overall mean calcium value observed during this study was 12.31 ± ৩.৫৩. The mean serum calcium values observed for various gestational age groups i.e. preterm, full term and post term were 10.80 ± 4.33, 12.44 ± 3.81 and 14.95 ± 1.60 respectively.

Serum inorganic phosphorus values :

Table - 19

Showing mean ± S.D. values of serum inorganic phosphorus (mg / dL), sex-wise

Sl. No.	Sex	No.of cases	Inorganic phosphorus (Mean ± S.D.)
1.	Male	19	6.68 ± 1.33
2.	Female	19	6.68 ± 1.83

't' = 0.33

'p' = $\angle$ 0.8

d.f. = 36

Table 19 denotes the mean value of serum inorganic phosphorus in different sexes of the study group. There was no difference in the mean value of cord blood inorganic phosphorus between male and female babies (6.68 mg%) and (6.68 mg%).

Table - 20

Showing mean ± S.D. inorganic phosphorus in different birth weight groups (mg/dL)

Sl. No.	Birth weight (gms)	No.of cases	inorganic phosphorus (Mean ± S.D.)
1.	< 2500	08	6.64 ± 1.91
2.	2500 - 3000	29	6.80 ± 1.88
3.	> 3000	01	6.67 ± 0.00

On comparison of mean serum inorganic phosphorus value in low birth weight babies with normal weight babies (table - 20), it was observed that cord blood inorganic phosphorus was lower (6.64 mg%) in low birth weight babies than in normal weight babies (6.80 mg%).

Table - 21

Showing mean ± S.D. values of serum inorganic Phosphorus levels (mg/dL) in various gestational age group

Sl. No.	Group	No.of cases	Serum inorganic phosphorus (Mean ± S.D.)
1.	Pre-term	04	6.42 ± 1.22
2.	Full term	32	6.67 ± 1.94
3.	Post term	02	7.45 ± 0.85

The overall mean inorganic phosphorus value observed during this study was 6.67 ± 1.59. The mean serum inorganic phosphorus values observedfor various gestational age groups i.e. pre-term, full term and post term were 6.42 ± 1.22, 6.67 ± 1.94 & 7.45 ± 0.85 respectively.

Serum-creatinine levels :

Table - 22

Showing mean ± S.D. values of serum creatinine (mg/dL), sex-wise

Sl. No.	Sex	No.of cases	Creatinine (Mean ± S.D.)
1.	Male	17	1.58 ± 0.35
2.	Female	18	1.61 ± 0.39

't' = 0.25

'p' = < 0.8

d.f. = 33

Table 22 denotes the mean value of serum creatinine in different sexes of the study group. It was observed that cord blood creatinine level was higher in female (1.6 mg%) than in male babies (1.58 mg%).

Table - 23

Showing mean ± S.D. serum-creatinine in different birth weight group (mg/dL)

Sl. No.	Birth weight (gm)	No.of cases	Serum creatinine (Mean ± S.D.)
1.	⟨ 2500	07	1.85 ± 0.22
2.	2500 - 3000	27	1.49 ± 0.21
3.	⟩ 3000	01	1.50 ± 0.00

On comparison of the mean serum creatinine value in low birth weight group with normal weight group (table - 23), it was observed that cord blood creatinine level was higher (1.85 mg%) in low birth weight group than in normal weight group (1.49 mg%).

Table - 24

Showing mean ± S.D. values of serum creatinine levels in various gestational age groups

Sl. No.	Groups	No.of cases	Serum creatinine (Mean ± S.D.)
1.	Pre-term	04	1.87 ± 0.21
2.	Full term	29	1.58 ± 0.37
3.	Post term	02	1.25 ± 0.25

The overall mean creatinine value observed during this study was 1.6 ± 0.35. Mean serum creatinine values observed in various gestational age group i.e. pre-term, full term and post term were 1.87 ± 0.21, 1.58 ± 0.37 and 1.25 ± 0.25 respectively.

Serum S.G.P.T. values :

Table - 25

Showing mean ± S.D. values of Serum - S.G.P.T. (IU/L), Sex-wise

Sl. No.	Sex	No.of cases	S.G.P.T. (Mean ± S.D.)
1.	Male	21	28.23 ± 10.02
2.	Female	24	26.95 ± 11.70

't' = 0.38

'p' = $\angle$ 0.7

d.f. = 43

Table 25 denotes the mean value of serum SGPT in different sexes of the study. It was observed that cord blood SGPT was higher in male (28.23 IU/L) than in female babies (26.95 IU/L).

Table - 26

Showing mean ± S.D. SGPT in different birth weight groups (IU/L)

Sl. No.	Birth weight (gm)	No.of cases	S.G.P.T. (Mean ± S.D.)
1.	⟨ 2500	10	29.50 ± 9.20
2.	2500 - 3000	33	27.17 ± 7.35
3.	⟩ 3000	02	24.00 ± 4.00

On comparison of the mean SGPT value in low birth weight group with normal weight group (table-26), it was observed that cord blood SGPT level was higher (29.50 IU/L) in low birth weight group than in normal weight group (27.17 IU/L).

Table - 27

Showing mean ± S.D. values of SGPT levels in various gestational age group (IU/L)

Sl. No.	Group	No.of cases	S.G.P.T. (Mean ± S.D.)
1.	Pre-term	05	24.00 ± 8.00
2.	Full term	38	27.89 ± 10.81
3.	Post term	02	30.00 ± 10.00

The overall mean SGPT value observed during this study was 27.55 ± 10.02. The mean serum SGPT values observed for various gestational age groups i.e. preterm, full term and post term were 24 ± 8, 27.89 ± 10.81, and 30 ± 10 respectively.

Serum-bilirubin value :

Table - 28

Showing mean ± S.D. values of bilirubin (mg/dL), sex-wise

Sl. No.	Sex	No.of cases	Bilirubin (Mean ± S.D.)
1.	Male	21	0.47 ± 0.23
2.	Female	23	0.51 ± 0.21

't' = 0.66

'p' = $\angle$0.5

d.f. = 42

Table 28 denotes the mean value of serum bilirubin in different sexes of the study. It was observed that cord blood bilirubin was higher in female (0.51 mg %) than in male babies (0.47 mg %).

Table - 29

Showing mean ± S.D. bilirubin in different birth weight groups (mg/dL)

Sl. No.	Birth weight (gms)	No.of cases	Bilirubin (Mean ± S.D.)
1.	⩽ 2500	08	0.64 ± 0.45
2.	2500 - 3000	33	0.48 ± 0.20
3.	⩾ 3000	03	0.46 ± 0.16

On comparison of the mean of serum bilirubin value in LBW babies with normal weight babies (table-29), it was observed that cord blood bilirubin value was higher (0.64 mg %) in the LBW group than in the normal birth weight group (0.48 mg %).

Table - 30

Showing mean ± S.D. values of bilirubin levels in maturity groups (gestational age)

Sl. No.	Group	No.of cases	Serum-bilirubin (Mean ± S.D.)
1.	Pre-term	04	0.51 ± 0.54
2.	Full-term	38	0.52 ± 0.22
3.	Post-term	02	0.35 ± 0.00

The overall mean bilirubin value observed during the study was 0.46 ± 0.23. The mean bilirubin value observed for various gestational age groups i.e. preterm, full term, and post term were 0.51 ± 0.54, 0.52 ± 0.22 and 0.35 respectively.

DISCUSSION

DISCUSSION

The present study was carried out to determine the normal serum levels of glucose, urea, creatinine, total proteins, albumin, calcium, inorganic phosphorus, aminotransferases and bilirubin in the cord blood of 49 newborn babies. The study was conducted at M.L.B. Medical College Hospital, Jhansi in the Department of Paediatrics from March, 1991 to February, 1992.

The primary aim of the study was to find out the normal biochemical values in newborn babies at birth and to establish the relationship, if any, of these biochemical values with birth weight and gestational age.

Besides evaluating biochemical values, weight was recorded and thorough physical examination of the newborn baby was done. Gestational age was calculated by counting the number of completed weeks from the first day of last menstrual period until the day baby was born.

Statistical analysis was done to derive mean $\pm$ S.D. values of glucose, urea, creatinine, total proteins, albumin, calcium, inorganic phosphorus, SGPT and bilirubin in male & female babies, in three gestational age groups (preterm, full term and post term) and birth weight groups (LBW and normal). Paired values were compared using the student t-test and significance of the differences (p values were noted.

Based on observation dipicted in various tables (1-30) inferences were drawn and these have been discussed in detail.

The sex of the child was given due consideration and accordingly the study group comprised on 25 (52.03%) male and 24 (48.97%) female cases (table - 1).

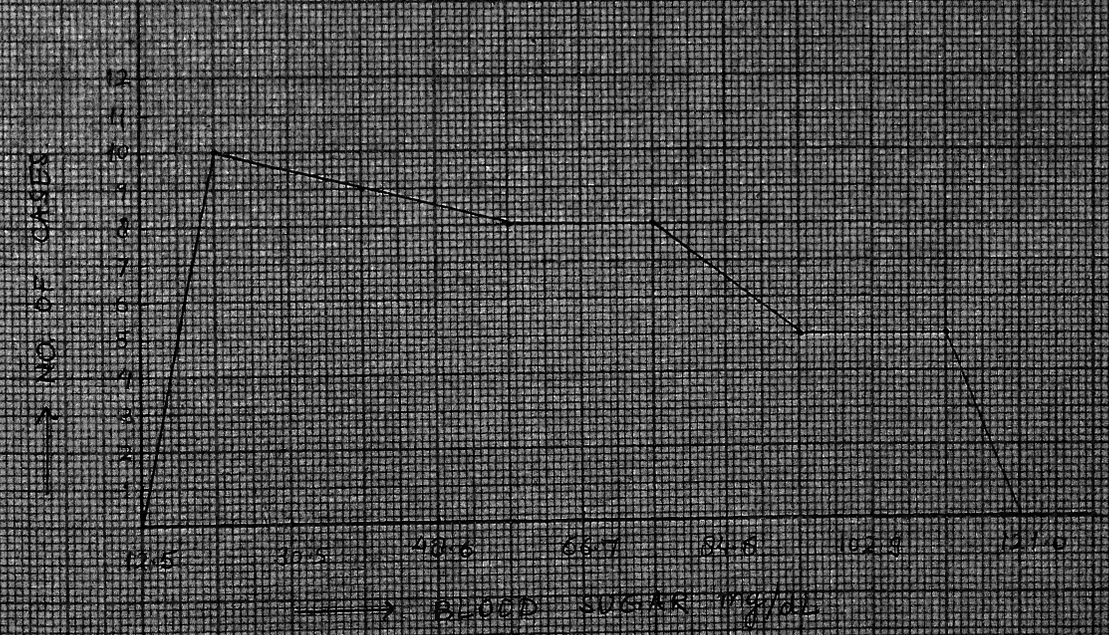
The birth weight was given its due importance and the study group was divided into low birth weight and normal birth weight groups as per the definition of low birth weight babies, WHO (1961). The low birth weight group included 10 (20.41%), while the normal weight group had 39 (79.59%) babies (table - 2). The percentage of low birth weight babies was low in the present study in comparison to normal birth weight babies. The cause may be that only those newborns, who were normally delivered, and whose mothers had no antenatal problem, were included in this study.

The study group was also divided into three groups preterm, full term and post term according to gestational age. The preterm included 05 (10.20%), full term 42 (85.71%) and post term group had 2 (4.08%) babies (table - 3).

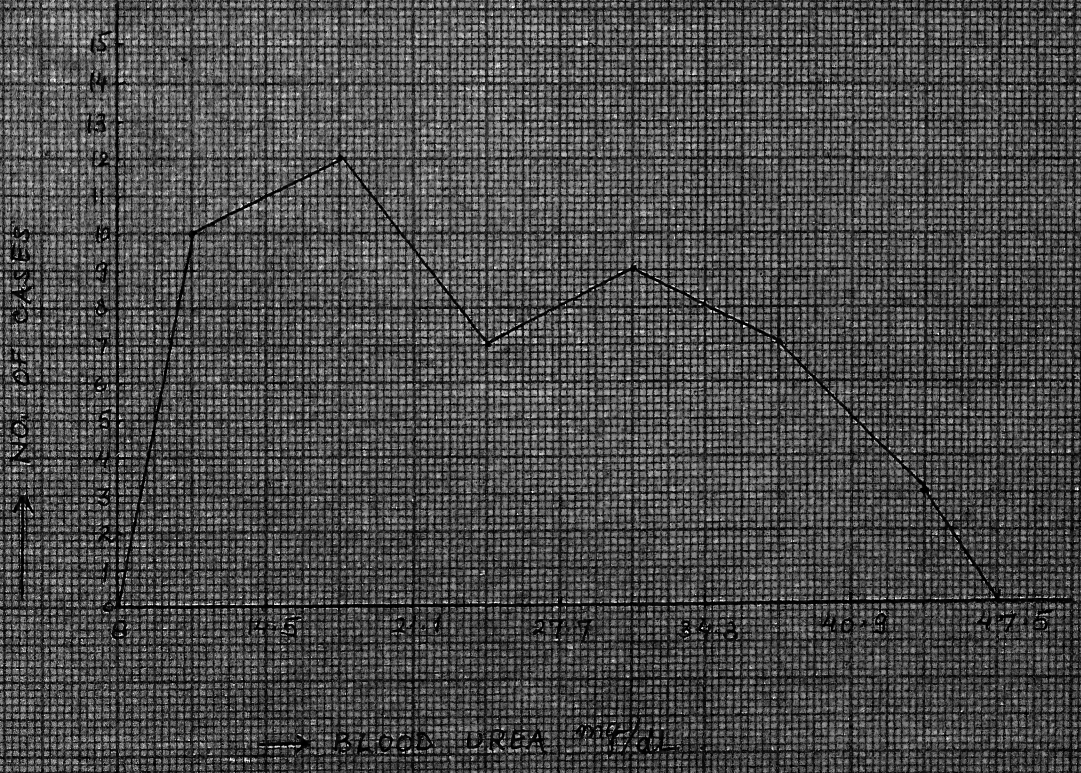
Frequency Distribution of various biochemical values in Newborns :

It shows the distribution of levels of blood sugar, blood urea, total serum proteins, serum albumin, serum

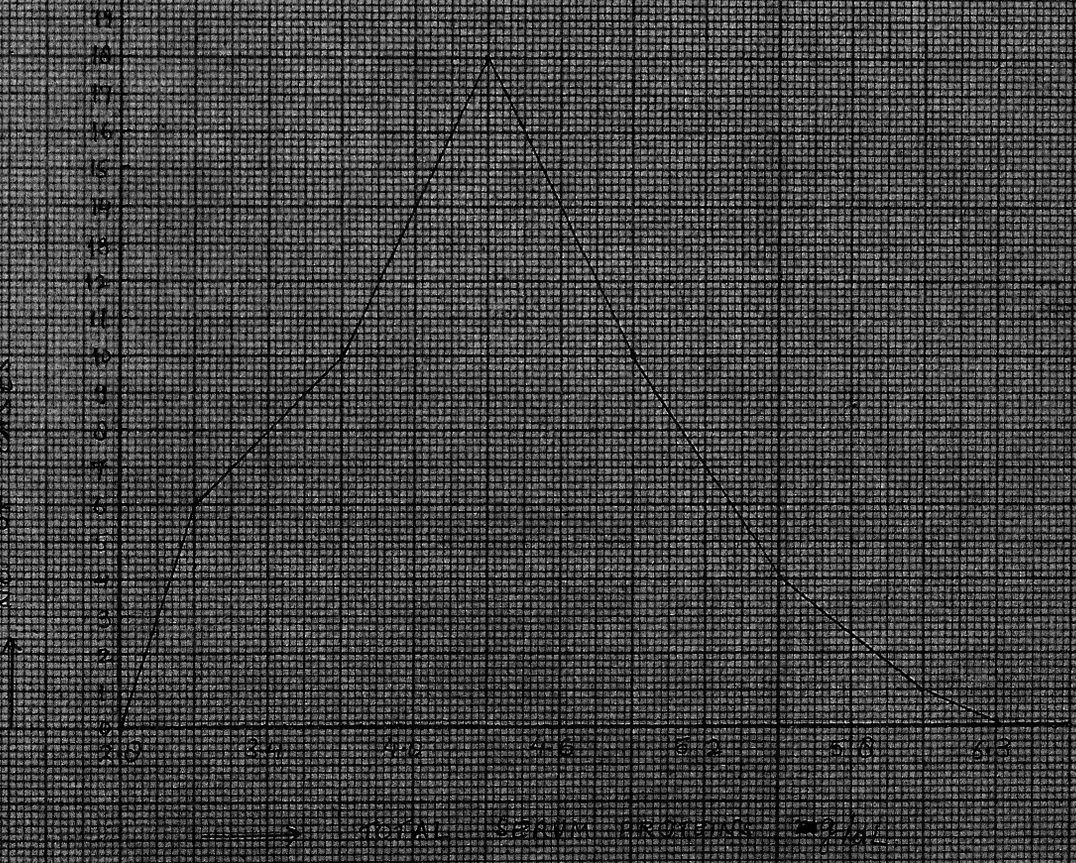
FREQUENCY DISTRIBUTION CURVE OF CORD BLOOD SUGAR IN NEWBORNS



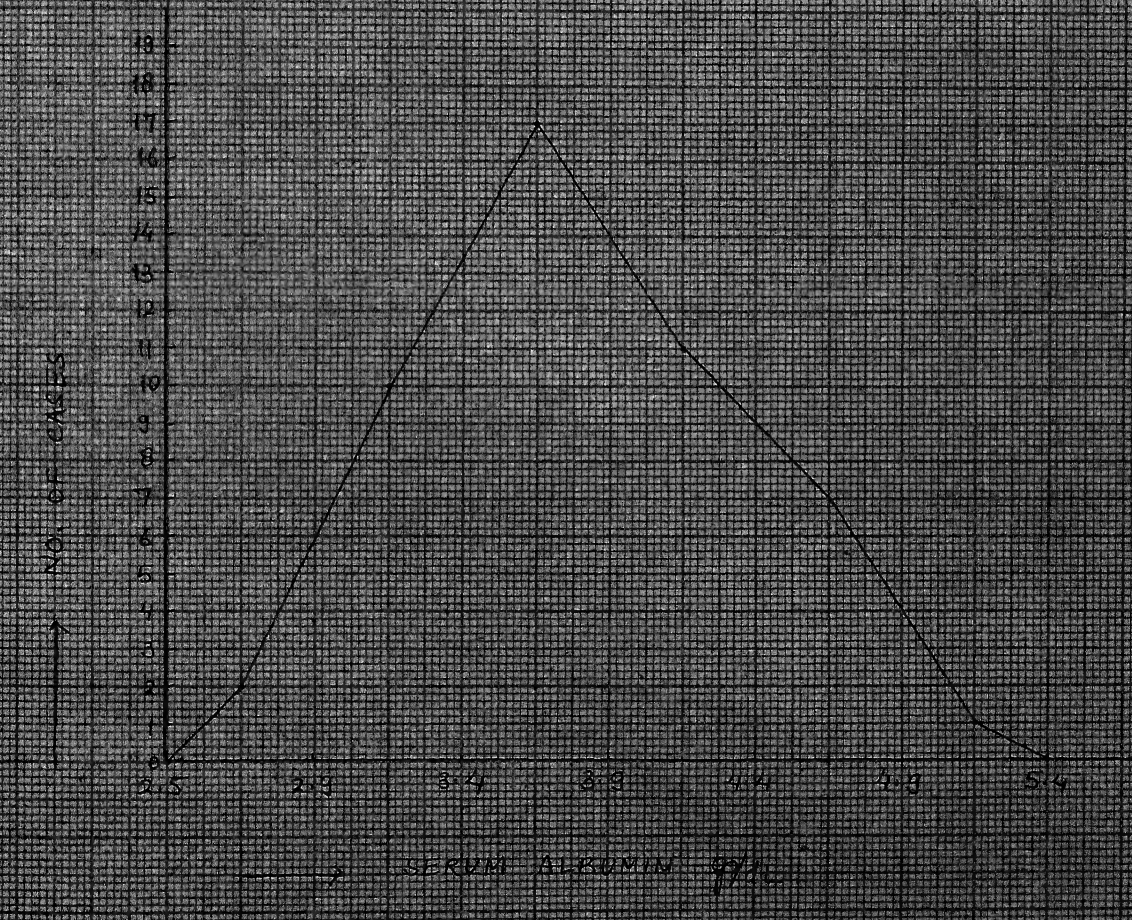
FREQUENCY DISTRIBUTION CURVE OF CORD BLOOD UREA IN NEWBORNS



FREQUENCY DISTRIBUTION CURVE OF CORD BLOOD TOTAL PROTEIN IN NEWBORNS



FREQUENCY DISTRIBUTION CURVE OF CORD BLOOD ALBUMIN IN NEWBORNS



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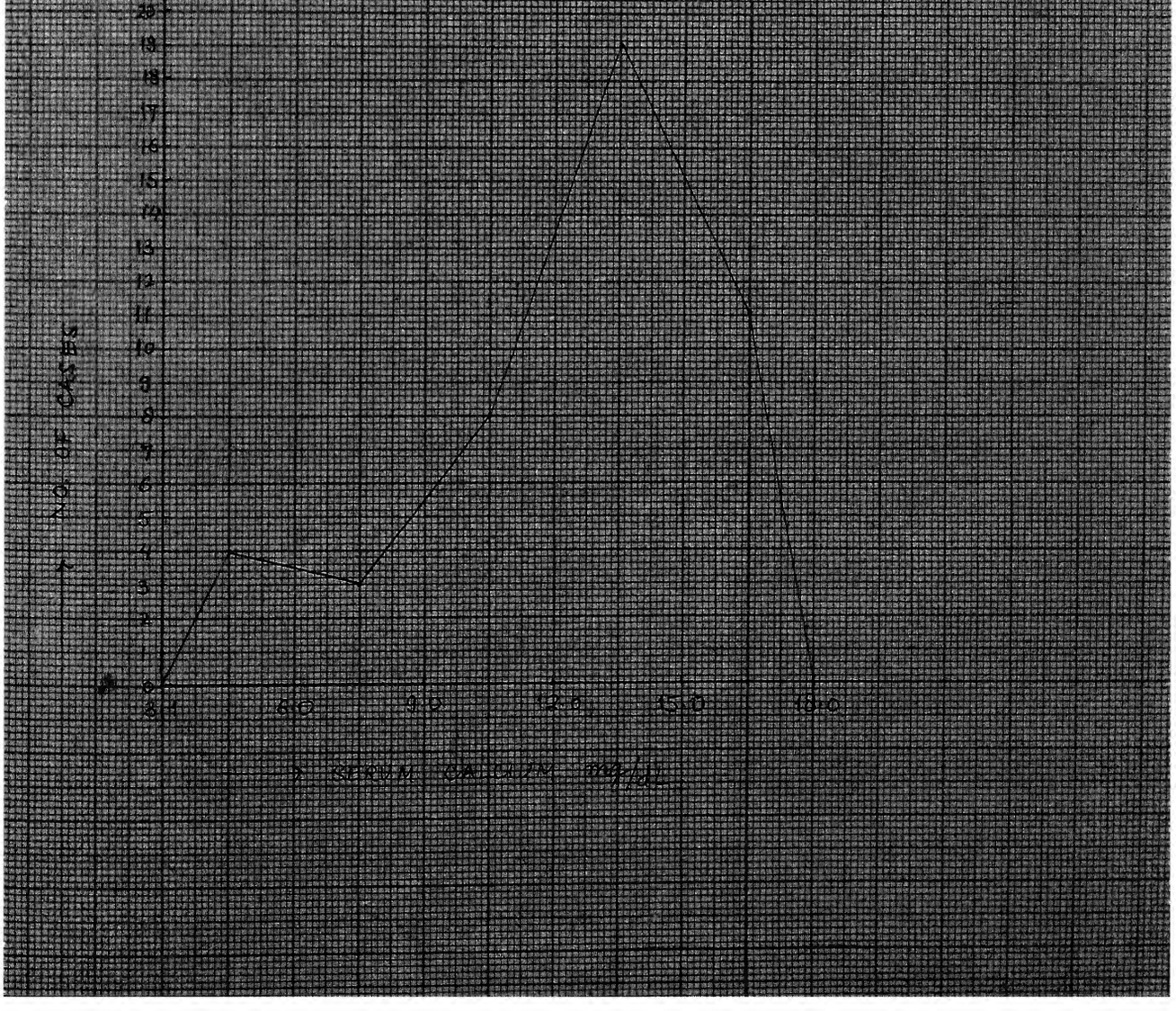

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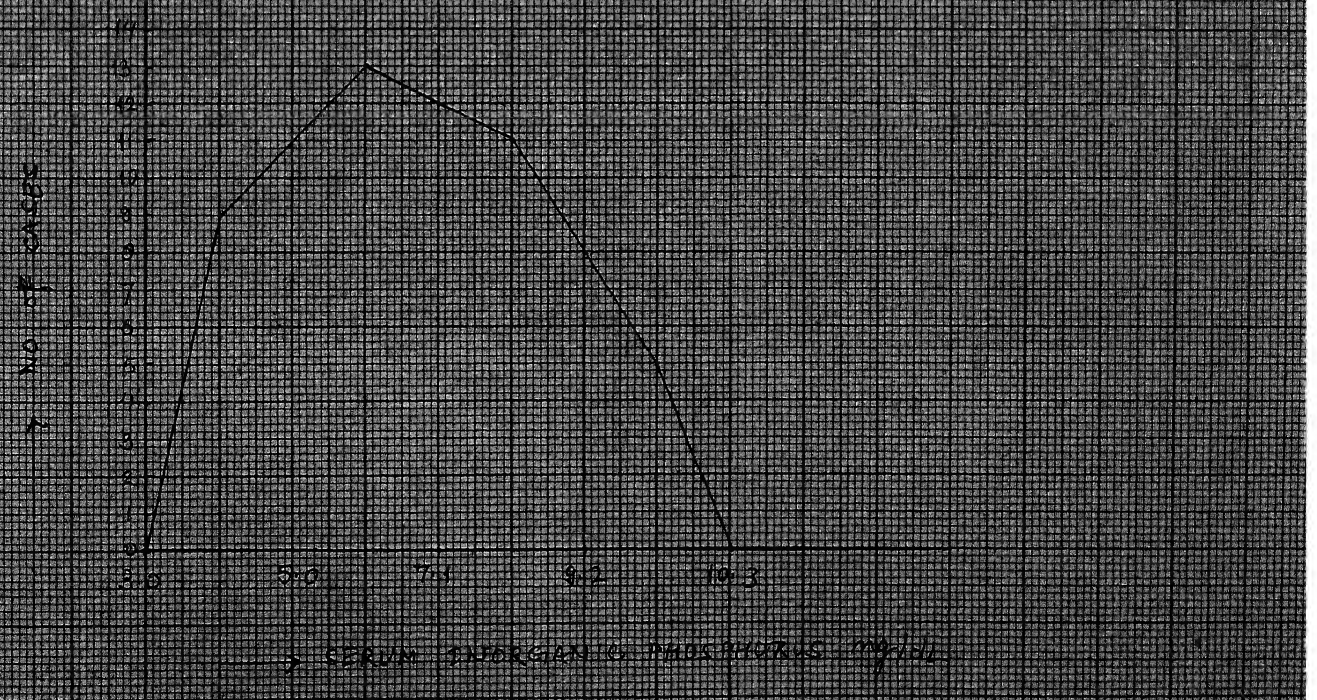
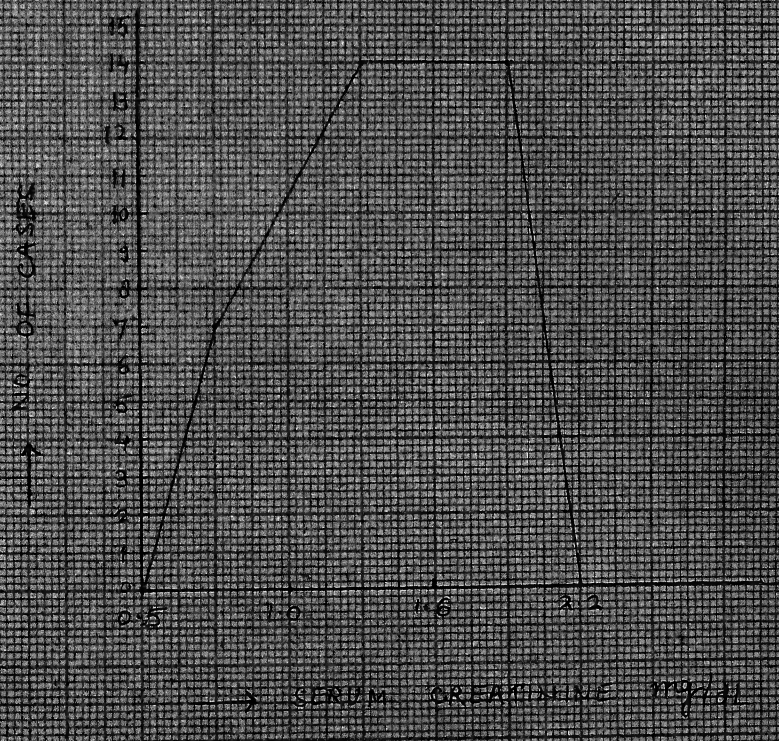
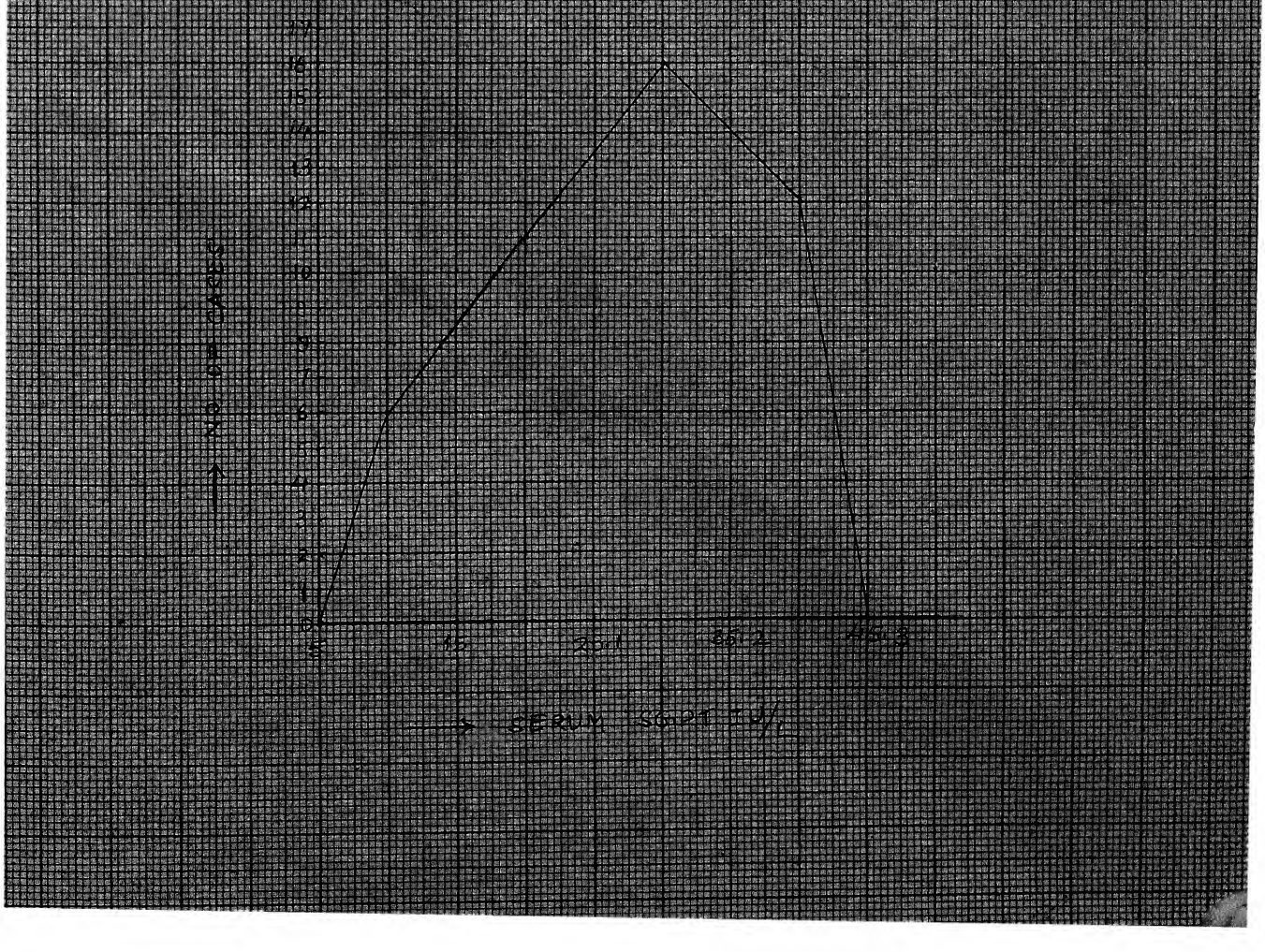


FREQUENCY DISTRIBUTION CURVE OF CORD BLOOD

CREATININE IN NEWBORNS

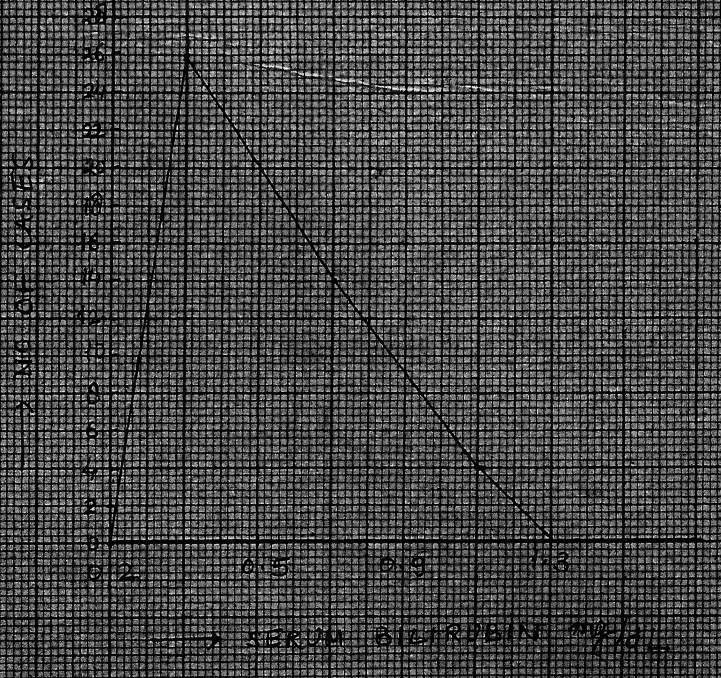


FREQUENCY DISTRIBUTION CURVE OF CORD BLOOD SGPT IN NEWBORNS



(23)

FREQUENCY DISTRIBUTION CURVE OF CORD BLOOD BILIRUBIN IN NEWBORNS



calcium, serum inorganic phosphorus, serum creatinine, serum SGPT and serum-bilirubin in study group (Graph 1 to 9).

The frequency distribution curves of blood sugar, total serum proteins, serum albumin, serum - inorganic phosphorus, serum-creatinine and serum SGPT show, normal distribution but the frequency distribution curves of blood urea, and serum-bilirubin show positive skewness (the curve is shifted to Right side) and the frequency distribution curve of serum calcium shows negative skewness (the curve is shifted to Left side) and peaked kurtosis. It means that the serum calcium level in the study group has more cases in the range of 12-15 mg/dL and less cases in the range of 3-9 mg/dL.

Statistics regarding the frequency distribution of various Biochemical values in the newborns.

Sl. No.	Biochemicals	No.of cases n	Mean $\bar{x}$	S.D. 'S'	For normal values Skewness (S)	Kurtosis (K)
1.	Blood sugar	45	63.08	22.84	0.28	1.80
2.	Blood Urea	48	23.08	11.80	0.73	2.42
3.	Total serum proteins	49	04.14	0.69	0.19	2.70
4.	Serum-albumin	48	03.65	0.45	0.13	2.48
5.	Serum calcium	45	12.31	03.60	- 0.98	3.32
6.	Serum inorganic phosphorus	38	06.67	01.50	0.16	1.97
7.	Serum creatinine	35	01.60	0.35	- 0.34	1.85
8.	Serum SGPT	45	27.55	10.02	- 0.31	2.05
9.	Serum bilirubin	44	0.46	0.23	0.96	2.77

Serum Glucose -

The overall mean value of glucose, irrespective of birth weight, gestational age and sex was 63.08± 22.84 mg/dL. In contrast to this value, Acharya and Payne (1965) observed a higher mean glucose level (73.0 mg/dL) in the cord blood. Similar to present study, Bhalla et al (1977) observed the mean glucose level at birth as 53.3 mg%.

(a) Relation with Sex :

In the present study female showed slightly higher level of glucose in their blood as compared to male babies, but on statistical analysis the difference did not reach a level of significance ($p > 0.6$) (table-4). Similar was the observation of McKittrick (1940); that males have slightly lower values in the first 24 hours. It has also been mentioned by Pildes et al (1967) that hypoglycaemic infants are predominantly males.

That mean blood glucose values are higher in female babies as compared to males, was also observed by Bhalla et al (1977).

(b) Relation with birth weight :

The relation of cord blood glucose with birth weight was studied by observing its variation in the normal and low birth weight babies.

On comparison, the glucose level was observed as to be lower in low birth weight group (57.80 mg%) than in the normal birth weight group (62.74 mg%), but the difference was not statistically significant ($p > 0.7$) (table - 5).

Similar to present study, Haworth et al (1967) has also observed that there was no variation in serum glucose level according to birth weight.

Bhalla et al (1977) has observed that babies less than 1000 gms had lower value and thereafter, the glucose value did not show any definite variation according to birth weight.

(c) Relation with gestational age :

In the present study serum glucose value was observed as 82.31 $\pm$ 34.84, 61.97 $\pm$ 29.94 and 76.05 $\pm$ 33.95 in preterm, full term and post term babies (table-6). The cord glucose level was observed higher in preterm (82.31 mg%) in comparison to full term (61.97 mg%) and post term (76.05 mg%) babies.

Table - 31

Showing the significance of difference between the paired values of mean cord blood glucose in preterm (I), full term (II) and post term (III) babies.

Sl. No.	Compared groups of table - 6	d.f.	't'	'p'
1.	I & II	41	2.81	p<.01
2.	II & III	38	0.61	p<0.6
3.	I & III	05	0.15	p<0.9

d.f. = degree of freedom.

In constrast to the present study Cornblath et al (1959), Brown and Wallia (1963) and Haworth (1965) observed that blood glucose during the first few days of life in premature babies ranged between 30 and 60 mg%.

Similar results were also observed by Shelly and Neligan (1966).

Vegad et al (1974) studied serum glucose levels in newborns and found lower levels of serum glucose in prematures (77.40 mg%) in comparison to full term babies (84.48 mg%).

Bhalla et al (1977) reported on serum glucose levels in preterm babies. They found lower value of serum glucose in preterm babies (45 mg%).

As regards, the raised levels of glucose in preterm babies, found in the present study, a possible explaination could be that all preterm deliveries were accomplished while using intravenous infusion (dextrose), to reduce the risk of foetal distress and consequent birth of still born babies.

Serum urea -

The overall mean value of urea, irrespective of birth weight, gestational age and sex, observed in the present study, was 23.08 ± 11.80 mg/dL.

In the past Acharya and Payne (1965) reported the mean level of urea in cord blood as being 29.37 ± 7.3 mg/dL, which is in close proximity with the result of present study. In contrast to present study, McCance et al (1947) observed a mean blood urea level of 18.9 mg/dL.

(a) Relation with Sex :

In the present study, there was only a slight difference in the urea levels of cord blood in male (23.08 mg%) and female babies (24.53 mg%). The difference was statistically not significant ($p > 0.6$) (table - 7).

(b) Relation with birth weight :

On comparing the mean cord blood urea levels of normal birth weight and low birth weight babies of the present study, the difference was statistically not significant ($p > 0.6$) (table - 8).

(c) Relation with gestational age :

In the present study serum urea levels as observed in preterm, full term and post term babies were 23.19 ± 10.39, 24.82 ± 10.62 and 21.00 ± 3.0 (table-9). The cord urea level was somewhat lower in post mature but the difference in each group was statistically not significant as shown in table - 32.

Table - 32

Showing the significance of difference between paired values of mean cord blood urea in pre-term (I), full term (II) and post term (III) babies.

Sl. No.	Compared groups of table - 9	d.f.	't'	'p'
1.	I & II	44	0.41	> 0.7
2.	II & III	41	1.66	> 0.1
3.	I & III	05	0.11	> 0.9

d.f. = degree of freedom.

Vegad et al (1974) has observed mean urea levels of 23.52 ± 4.78 and 23.20 ± 4.56 in preterm and full term babies respectively. The observations of present study are in close proximity to those of the above mentioned workers and as it is apparant that hardly any difference existed in the urea levels of preterm and full term babies in both the studies.

Total serum proteins :

The overall mean value of total serum protein (irrespective of birth weight, gestational age and sex) was 4.14 ± 0.69 g/dL, in the present study.

Higher cord blood total serum protein level (6.13 ± 0.67 g/dL) was observed by Acharya and Payne (1965) in their study.

Similar to the study of Acharya et al (1965), Sitadevi (1969) observed higher cord blood protein level (6.13 ± 0.62 mg%) as compared to present study.

The value of total serum protein reported by Vegad et al (1974) (5.6 ± 0.36) is higher in comparison to the level observed in present study.

The discrepancy observed between the results of various authors is probably due to the difference in methods used for the estimation of total serum proteins.

(a) Relation with Sex :

Analysing the variation of total protein with the sex of child, it was found that females had slightly higher total serum protein level (4.11 gm%) than male babies (4.01 gm%). However, the difference was not significant ($p > 0.7$) (table - 10).

Sitadevi (1969), also, did not observe sex difference in total serum protein levels of newborn babies.

(b) Relation with birth weight :

Total serum protein level observed in the present study was lower in low birth weight babies (3.5 mg%) than in normal birth weight babies (4.3 mg%) and the difference was statistically significant $p < .001$ (table - 11).

Similar results were also observed by Rapoport et al (1943). They observed that total serum protein levels were lower in the low birth weight babies (4.6 gm%) than in normal birth weight babies (5.1 gm%).

Sitadevi (1969) observed that there was absolutely no correlation between the birth weight and total serum proteins.

Similar to the present study Vegad et al (1975) observed that total serum protein level was lower in low birth weight (4.4 g%) than in normal birth weight babies (5.6 gm%).

(c) Relation with gestational age :

In the present study total serum protein values were observed as 3.32 ± 0.30, 4.25 ± 0.67 and 4.00 ± 0 in preterm, full term and post term babies (table - 12). The cord blood total serum protein value was lower in preterm (3.31 gm%) than in full term (4.25 gm%) and post term babies (4.0 gm%) babies.

Table - 33

Showing the significance of difference between paired values of mean cord blood total serum proteins in preterm (I), full term (II) and post term (III) babies.

Sl. No.	Compared groups of table-12	d.f.	Total serum protein 't'	'p'
1.	I & II	45	3.00	<0.01
2.	II & III	42	0.58	<0.60
3.	I & III	05	2.80	<0.05

A statistically significant difference was observed in preterm (I) and full term (II) babies ($p<0.01$). The probable cause could be immature liver functions in preterm babies.

Darrow and Cary (1933) observed a lower serum protein concentration in preterm (4.9 gm%) than in full term babies (5.52%). This observation was in agreement with the observation from the present study.

Similar results were observed by Hickman et al (1943). A lower total serum protein level in preterm (3.7 - 5.4 gm%) than full term babies (4-7 gm%) was observed by the authers during the first four weeks of human life.

Sitadevi (1969) has also observed that the total serum protein value in preterm was slightly lower than that observed in full term babies.

Vegad et al (1975) has also observed significant difference in total serum protein of preterm (4.79%) and full term babies (5.67 gm%).

Serum - albumin :

The overall mean value of serum albumin (irrespective of birth weight, gestational age and sex) as observed in the present study was 3.65 $\pm$ 0.45 g/dL.

The mean value of serum albumin (3.79 $\pm$ 0.53 gm%) reported by Trevorrow et al (1942) is in close proximation to the value observed in the present study.

Similar results were also reported by Sitadevi (1969). She reported mean serum albumin level of 3.37 ± 0.48 g/dL in the cord blood of newborns.

Relation with sex :

In the present study no difference in cord blood albumin level, between male (3.72 g%) and female babies (3.72 g%), was seen.

Relation with birth weight :

In present study, on comparing the cord blood albumin level of normal birth weight babies (3.91 ± 0.58 gm%) with low birth weight babies (3.3 ± 0.35 gm%), a lower albumin value was observed in low birth weight babies and the difference was statistically significant ($p < .001$) (table - 14).

In contrast to observation from the present study, Sitadevi (1969) observed no significant correlation between serum albumin and birth weight.

Relation with gestational age :

In the present study mean serum albumin value was 3.15 ± 0.43, 3.80 ± 0.61 and 4.00 ± 0 in preterm, full term and post term babies respectively. The cord albumin level was observed (table 15) as lower in preterm (3.15 gm%) than in full term (3.80 gm%) and post term (4.00 gm%) babies with a statistically significant difference as shown in table 34.

Table - 34

Showing the significance of difference between paired values of mean cord blood albumin in preterm (I), full term (II) and post term (III) babies.

Sl. No.	Compared groups of table 15	d.f.	Serum-albumin 't'	'p'
1.	I & II	44	2.32	<0.05
2.	II & III	41	0.46	<0.7
3.	I & III	05	2.57	<0.05

d.f. = degree of freedom.

A significant difference was observed in preterm (I) and full term (II) babies ($p<0.05$). The probable cause could be the immature liver function in preterm babies.

Similar results were reported by Sitadevi (1969) in her study. She reported lower cord albumin value (3.15 gm%) in preterm than in full term babies (3.37 gm%). But, there was no significant correlation between the gestational age and serum albumin value.

Serum Calcium :

The overall mean value of calcium (irrespective of birth weight, gestational age and sex) was (12.31 $\pm$ 3.60 mg/dL) in the present study.

In contrast to the present study, value of mean serum calcium reported by Acharya and Payne (1965) was lower (9.4 mg/dL) than observed in the present study.

Vegad et al (1975) reported a mean serum calcium level of 10.00 $\pm$ 2.8 mg/dL at birth.

Relation with sex :

In the present study male babies showed a slightly lower calcium level (11.93 mg%) than female babies (12.85 mg%) but on statistical analysis the difference was not significant ($p > 0.5$) (table - 16).

Relation with birth weight :

In the present study serum calcium level was observed lower in low birth weight (11.20 mg%) than in normal birth weight babies (12.90 mg%) but the difference was not statistically significant ($p > 0.4$) (table - 17).

A significant difference between the mean calcium levels of low birth weight and normal birth weight has been reported by Gittleman et al (1956).

Similar to present study values of serum calcium reported by Vegad et al (1975) are lower in low birth weight (8.3 mg%) than normal birth weight babies (10.0 mg%).

Relation with gestational age :

In the present study following serum calcium values were observed :

Preterm 10.8 ± 4.33; full term 12.44 ± 3.81, post term 14.95 ± 1.60 mg% respectively (table 18).

The cord calcium level was observed lower in preterm (10.8 mg%) than full term (12.44 mg%) and post term (14.95 mg%) babies (but difference between these groups was statistically not significant as shown in table - 35).

Table - 35

Showing the significance of difference between paired values of mean cord blood calcium in preterm (I), full term (II) and post term (III) babies.

Sl. No.	Compared groups of table 18	d.f.	Serum-calcium 't'	'p'
1.	I & II	41	0.82	⟨0.5
2.	II & III	39	0.99	⟨0.4
3.	I & III	04	1.09	⟨0.4

d.f. = degree of freedom.

The mean calcium value reported by Vegad et al (1975) was lower in preterm (9.01 ± 3.34 mg%) than full term (10.00 ± 2.81 mg%) babies, a finding which is similar to the present study.

The mean value of calcium in preterm is lower, probably due to the fact that active bone formation takes place between 36 and 40 weeks of pregnancy.

Serum Inorganic Phosphorus :

The overall mean value of inorganic phosphorus observed in the cord blood (irrespective of birth weight gestational age and sex) was 6.67 $\pm$ 1.50 mg/dL in the present study.

A slightly lower value (5.65 $\pm$ 1.28 mg/dL) of inorganic phosphorus was reported by Acharya and Payne (1965).

Relation with sex :

In the present study, no significant difference was observed ($p > 0.8$) in serum inorganic phosphorus between the male (6.68 mg%) and female babies (6.68 mg%).

Relation with birth weight :

There was no much difference in mean inorganic phosphorus level between the normal and low birth weight babies and the difference was also statistically not significant ($p > 0.9$) (table-20).

Relation with gestational age :

In the present study following serum inorganic phosphorus value was observed :

Pre-term 6.42 ± 1.22; full term 6.76 ± 1.94 ; and post term 7.45 ± 0.85 mg/dL respectively (table 21).

The cord blood inorganic phosphorus was observed higher in full term(6.76 mg%) and post term (7.45 mg%) than preterm (6.42 mg%). On statistical analysis the difference in mean inorganic phosphorus level between preterm, full term and post term was not significant as shown in table - 36.

Table - 36

Showing the significance of difference between paired values of mean cord blood inorganic phosphorus in preterm (I), full term (II) and post term (III) babies.

Sl. No.	Compared groups of table 21	d.f.	Serum-inorganic phosphorus 't'	'p'
1.	I & II	34	0.35	<0.8
2.	II & III	04	0.95	<0.4
3.	I & III	32	0.53	<0.6

Serum creatinine :

The overall mean value of serum creatinine (irrespective of birth weight gestational age and sex) was 1.6 ± 0.39 mg/dL in the present study.

Reference ranges of serum creatinine are lacking in neonates.

Relation with sex :

In the present study observed higher cord blood creatinine level in female (1.61 ± 0.39 mg%) than male babies (1.58 ± 0.35 mg%) but the difference in mean creatinine level between male and female babies was statistically not significant ($p > 0.8$) (table - 22).

Relation with birth weight :

In the present study, the cord blood creatinine was observed higher in low birth weight (1.85 mg%) than normal birth weight babies (1.49 mg%) and difference in creatinine value between LBW and normal weight babies was statistically significant ($p < 0.1$) (table - 23).

Stonestreet et al, 1978 also reported higher value 1.3 ± 0.07 mg/dL in low birth weight babies which stablished at 0.6 ± 0.05 mg% during the second and third month of life.

As regards the raised level of creatinine in LBW babies found in the present study, a possible cause could be that all LBW babies have poor renal function.

Relation with gestational age :

In the present study following cord blood creatinine value was observed - 1.87 ± 0.21, 1.58 ± 0.37, and 1.25 ± 0.25 mg/dL in preterm, full term and post term respectively,(table - 24). The cord blood creatinine level was observed higher in preterm (1.87 mg%) than full term (1.58 mg%) and post term (1.25 mg%).

Table - 37

Showing the significance of difference between paired values of mean cord blood creatinine in preterm (I), full term (II) and post term (III) babies.

Sl. No.	Compared groups of table 24	d.f.	Serum-creatinine 't'	'p'
1.	I & II	31	1.52	⟨0.1
2.	II & III	29	1.22	⟨0.2
3.	I & III	04	3.10	⟨0.5

d.f. = degree of freedom.

A statistically significant difference was observed in preterm (I) and full term (II) babies ($p < 0.1$). The probable cause could be poor renal perfusion in preterm babies.

Serum S.G.P.T. :

The overall mean value of SGPT (irrespective of birth weight, gestational age and sex) was 27.55 ± 10.12 IU/L in the present study.

Relation with sex :

In the present study female shows lower serum SGPT values (26.95 ± 11.70 IU/L) than male babies (28.23 ± 10.02 IU/L) but on statistical analysis the difference between mean values of SGPT between male and female babies was not significant ($p < 0.7$) (table-25).

Relation with birth weight :

In the present study on comparing the cord blood SGPT levels was observed (29.5 ± 9.20 IU/L) higher in low birth weight than normal birth weight babies (27.17 ± 7.35 IU/L) but on statistical analysis the difference was not significant ($p < 0.6$) (table - 26).

Relation with gestational age :

In the present study mean serum SGPT value was 24 ± 8, 27.89 ± 10.81, 30 ± 10 IU/L in preterm, full term and post term respectively (table - 27).

The cord SGPT value was observed as lower in preterm (24 IU/L) than full term (27.89 IU/L) and post term babies (30 IU/L) but on statistical analysis the difference between the mean value of SGPT level in preterm, full term and post term was not significant as shown in table - 38.

Table - 38

Showing the significance of difference between paired values of mean cord blood SGPT in preterm (I), full term (II) and post term (III) babies.

Sl. No.	Compared groups of table-27	d.f.	Serum S.G.P.T. 't'	'p'
1.	I & II	41	0.76	<0.5
2.	II & III	05	0.73	<0.5
3.	I & III	38	0.26	<0.8

d.f. = degree of freedom.

Serum bilirubin :

The overall mean value of serum bilirubin (irrespective of birth weight, gestational age and sex) was 0.46 $\pm$ 0.23 mg/dL in the present study.

Relation with sex :

In the present study there was only slightly higher serum bilirubin level in female (0.51 ± 0.21 mg%) than male babies (0.47 ± 0.23 mg%) and on statistical analysis the difference between mean value in male and female babies was not significant ($p > 0.8$) (table-28).

Relation with birth weight :

It was observed that cord blood bilirubin was higher in low birth weight (0.64 mg%) than normal weight babies (0.48 mg%) and on statistical analysis the difference between mean bilirubin level in LBW and normal birth weight babies was not significant $p > 0.8$ (table - 29).

Relation with gestational age :

In the present study mean serum bilirubin level was 0.51 ± 0.54, 0.52 ± 0.22, 0.35 mg/dL in preterm, full term and post term respectively. The cord bilirubin was observed no significant difference in these groups and on statistical analysis the difference between mean values of serum bilirubin in preterm, full term and post term was not significant ($p > 0.6$).

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SUMMARY AND CONCLUSION

SUMMARY & CONCLUSIONS

The present work was carried out to study the normal serum levels of glucose, urea, creatinine, total proteins, albumin, calcium, inorganic phosphorus, aminotransferases and bilirubin in cord blood of 49 newborn babies. The study was conducted at M.L.B. Medical College, Jhansi in the Department of Paediatrics, from March, 1991 to February, 1992.

The primary aim of the study was to find out the normal biochemical values in newborn babies at birth in cord blood and to establish the relationship, if any, of these biochemical values with birth weight, gestational age and sex of the newborn babies.

An attempt was made to compare the biochemical levels in low birth weight to normal birth weight babies.

Besides evaluating biochemical values, weight was recorded and thorough physical examination of the newborn baby was done. The gestational age was calculated by counting the number of weeks from the first day of last menstrual period till the date of birth of baby.

A total 49 cases were examined in this study. The cases were grouped into preterm, full term and post term groups on the basis of gestational age.

On the basis of birth weight cases were divided into low birth weight and normal birth weight group as per definition of low birth weight babies given by WHO (1961). The low birth weight group included 10 (20.41%), while the normal weight group had of 39 (79.59%) babies included and observed different biochemical values.

The sex of the child was given due consideration and accordingly, the study group comprised of 25 (52.03%) male and 24 (48.97%) female cases.

1. Normal Biochemical levels :

In the present study the mean values of glucose, urea, total proteins, albumin, calcium, inorganic phosphorus, creatinine, bilirubin and SGPT, irrespective of birth weight, gestational age and sex were observed as -

Serum glucose	-	63.08 ± 22.84 mg/dL
Serum urea	-	23.08 ± 11.80 mg/dL
Total serum protein	-	04.14 ± 0.69 g / dL
Serum albumin	-	03.65 ± 0.45 g /dL
Serum creatinine	-	01.60 ± 0.35 mg/dL

Serum calcium	-	12.31 ± 3.60 mg/dL
Serum inorganic phosphorus	-	06.67 ± 1.50 mg/dL
Serum bilirubin (total)	-	0.46 ± 0.23 mg/dL
Serum SGPT	-	27.55 ± 10.02 IU/L

2. Relation with maturity of newborn :

The variation in biochemical values according to the gestational age was follows :

Sl. No.	Biochemicals	Preterm	Full-term	Post-term
1.	Serum glucose mg/dL	82.31 ± 34.84	61.97±29.94	76.05±33.95
2.	Serum urea mg/dL	23.19 ± 10.39	24.82±10.39	21.00±03.00
3.	Serum total protein mg/dL	03.32 ± 0.30	04.25± 0.67	04.00±00
4.	Serum albumin g/dL	03.15 ± 0.43	03.80± 0.61	04.00±00
5.	Serum calcium mg/dL	10.80 ± 04.33	12.44±03.81	14.95±01.60
6.	Serum inorganic phosphorus mg/dL	06.42 ± 01.22	06.76±01.94	07.45± 0.85
7.	Serum creatinine mg/dL	01.87 ± 0.22	01.58± 0.37	01.25± 0.25
8.	Serum bilirubin	0.51 ± 0.54	0.52± 0.22	0.35±00
9.	Serum SGPT	24.00 ± 08.00	27.89±10.81	30.00±10.00

There was no significant variation observed in any of the biochemical values according to the maturity status.

3. Relation with birth weight :

Significant difference was observed in total protein, albumin, creatinine levels in cord blood of LBW group as compared with the normal weight group.

	LBW	NBW
Total serum protein g/dL	3.54 ± 0.46	4.30 ± 0.65
Serum albumin g/dL	3.30 ± 0.35	3.90 ± 0.54
Serum creatinine mg/dL	1.85 ± 0.22	1.50 ± 0.50

4. Relation with sex :

There was no significant difference in any of the biochemical values according to the sex of baby.

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BIBLIOGRAPHY

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APPENDIX

STUDY OF BIOCHEMICAL VALUES IN NEWBORNS (Cord Blood)

Place of work : Immunology and Biochemistry Lab,
Department of Paediatrics,
M.L.B. Medical College, Hospital,
Jhansi.

Investigator : Dr. Shalini Sharma

Guide : Dr. Ramesh Kumar, MD, DCH,
Professor & Head,
Department of Paediatrics,
M.L.B. Medical College,
Jhansi.

CASE NO. ____

MRD NO. Date :

Name : Sex :

Date of birth : Weight at birth :

Mother's name : Age :

Father's name : Age :

Socio-economic status :

address :

Order of birth :

Gravida : Parity : Abortion:

BRIEF OBSTETRICAL HISTORY OF MOTHER

Gravida : Parity : Abortion:

Last menstrual period :

Gestational age :

Last child birth :

Previous evidences of bad obstetrical history (specify) :

Others :

ANTENATAL HISTORY

Fever

Rashes

Convulsions

Exposure to Radiations :

Maternal smoking

Drug intake, any

Pre-eclamptic or eclamptic toxemia

Antepartum haemorrhage (any trimester)

Leaking P/V -/12 hrs.

-/12 hrs.

Whether received supplementation of

- Iron
- Vitamins
- Minerals
- others

Chronic Diseases :

- Respiratory
- Cardiac
- Renal
- Metabolic
- Others

NATAL HISTORY

Presentation :

Mode of delivery : Normal/Forceps/Caessarian Vaginal

Any feature of birth asphyxia :

History of syntocinon drip :

Others (Drugs etc) :

DIETARY HISTORY OF MOTHER

Vegetarian / Non-vegetarian

Average intake of - Calries

- Proteins

EXAMINATION OF MOTHER

GENERAL EXAMINATION

General appearance	Hydration
Nutrition	Oedema
Built	Clubbing
Pulse Rate	Lymph Nodes
Resp. Rate	Skin
B.P.	Oral cavity

Anemia (Pallor)	Eyes
Icterus	Weight
Cyanosis	Height

SYSTEMIC EXAMINATION

Cardiovascular system

Respiratory system

Abdomen

Central Nervous system

EXAMINATION OF NEWBORN

I. GENERAL APPEARANCE

Activity :

Colour :

Cry :

Posture :

II. ANTHROPOMETRIC EXAMINATION

Weight

Length

Head circumference

Chest circumference

III. GENERAL EXAMINATION

Heart rate :	Eyes :
Resp. Rate :	Oral cavity :
Skin :	Neck & Trunk:
Head - A/F :	Umblicus :
Face :	Genitalia :
Scalp : Caput	Any other congenital anomaly
Succedanium	Others
Others	

IV. SYSTEMIC EXAMINATION

A. Cardiovascular system

B. Respiratory System

C. Abdomen

D. Neurological Examination

Neonatal reflexes :

Rooting reflex -

Sucking reflex -

Moro's reflex -

Reflex activity-

Any other -

INVESTIGATIONS

Sample collected on :

- NEWBORN

a. Cofd blood

Blood urea	Serum creatinine
Blood sugar	Serum Bilirubin
Serum Calcium	SGPT
Serum Albumin	Total Proteins :
Serum Phosphatase: (total)	A|G Ratio

b. Weight :

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